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(71) Applicant: IGEN, INC. [US/US]; 16020 Industrial Drive, Gaithersburg, MD 20877 (US).

(72) Inventors: BARD, Allen, J.; 6202 Mountain Climb Drive, Austin, TX 78731 (US). XU, Xiao-Hong; 918 East 40th Street #112, Austin, TX 78751 (US).

(74) Agent: RYAN, John, W.; Igen, Inc., Legal Dept., 16020 Industrial Drive, Gaithersburg, MD 20877 (US).

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(54) Title: BIOSENSOR FOR AND METHOD OF ELECTROGENERATED CHEMILUMINESCENT DETECTION OF NUCLEIC ACID ADSORBED TO A SOLID SURFACE

(57) Abstract

Single-strand DNA was immobilized on an electrode covered with an aluminum alkanebisphosphonate film by immersing it in an ss-DNA solution. The immobilized ss-DNA labeled with $Ru(bpy)_3^{2+}$ was detected by monitoring the electrogenerated chemiluminescence (ECL) produced upon oxidation in a solution containing tri-n-propylamine. After immobilization of unlabeled ss-DNA, a complementary labeled strand DNA was hybridized to produce ds-DNA on the surface. The extent of DNA hybridization was determined by ECL of the labeled $Ru(bpy)_3^{2+}$. Surface immobilized ds-DNA could also be detected by observing the ECL of intercalated $Ru(phen)_3^{2+}$. Transmission electron microscopy (TEM) was employed to image the film and the immobilized DNA.

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Title of the Invention

BIOSENSOR FOR AND METHOD OF ELECTROGENERATED CHEMILUMINESCENT DETECTION OF NUCLEIC ACID ADSORBED TO A SOLID SURFACE

Field Of Invention

The present invention relates to the diagnostic field, and especially nucleic acid diagnostics. In particular, the present invention relates to a probe or sensor having a film containing metal centers, its preparation where a single-strand or double-strand nucleic acid sequence is immobilized thereon on, and its use in the subsequent detection of the nucleic acid by labelling with luminescent metal chelates.

In addition to using the subject biosensor for nucleic acid diagnostics based on a surface designed modified electrical sensor, i.e., chips or electrode, immobilization and hybridization of nucleic acid such as DNA on a self-assembled thin film via surface reaction is also useful in studying molecular recognition of DNA.

Background Of Invention

Nucleic acid diagnostics has become an important area in molecular biology and biotechnology studies, with applications to the determinations of disease and food contaminating organisms and in forensic and environmental investigations. The development of new DNA biosensors has led to the application of several detection techniques such as

optical methods (e.g., luminescence, ellipsometry and pseudo-Brewster angle reflectometry), piezoelectric devices (e.g., SAW, QCM), and electrochemical techniques (e.g., CV and SWV). Of particular interest are labels which can be made to luminesce through photochemical, chemical, and electrochemical reaction schemes. In particular, electrochemiluminescent methods of determining the presence of labelled materials are preferred over other methods for many reasons. They are highly diagnostic of the presence of a particular label, sensitive, nonhazardous, inexpensive and can be used in a wide variety of applications. Suitable labels comprise electrochemiluminescent compounds, including organic compounds and metal chelates.

For example, electrochemiluminescent ruthenium- and osmium-containing labels have been used in methods for detecting and quantifying analytes of interest in liquid media (U.S. Patent Nos. 5,310,687; 5,238,808; and 5,221,605). In addition, the application of electrogenerated chemi-luminescence (ECL) measurements to the detection of solution phase DNA intercalated with ruthenium-containing labels has been described (Carter, M.T. et al. (1990) Bioconjugate Chem 2:257-263). However, reaction schemes that are viable in the solution phase are often not applicable in the solid phase. More importantly, the detection of solution phase analytes such as DNA has several drawbacks relative to detection of analytes absorbed to solid surfaces. The advantages for detecting DNA via solid phase techniques as opposed to

solution techniques are: (1) more sensitive (detection of monolayer quantities); (2) easier to separate DNA from sample (avoid interferences); and (3) possibility of detection of several different DNA in single analysis, with localized probes, e.g., as in sequencing studies.

These luminescent systems are of increasing importance in diagnostics. For example, in U.S. Pat. No. 4,372,745, chemiluminescent labels are used in immunochemical applications where the labels are excited into a luminescent state by reaction of the label with H_2O_2 and an oxalate. In these systems, H_2O_2 oxidatively converts the oxalate into a high energy derivative, which then excites the label. It is expected, that in principle, the H_2O_2 and an oxalate reaction scheme should work with any luminescent material that is stable under the oxidizing conditions of the assay, and can be excited by the high energy oxalate derivative. Unfortunately, this very versatility is a major drawback of the 4,372,745 patent: lack of selectivity or specificity, i.e., typical biological fluids containing the analyte of interest also contain a large number of potentially luminescent substances that can cause high background levels of luminescence.

Thus a need exists for a solid phase system, e.g., a biosensor, and method that (1) provides the necessary specificity absent systems that rely on the H_2O_2 and oxalate reaction scheme; and (2) do not depend on solution techniques. The present invention overcomes the limitations and drawbacks

of the prior art.

Summary Of Invention

The present invention provides a biosensor and its use for electrogenerated chemiluminescent detection of nucleic acid absorbed to a solid surface via the use of ruthenium- and osmium-containing chemiluminescent labels.

An object of the present invention is to provide a film containing an aluminum (III) alkanebisphosphonate layer having metallic aluminum centers, i.e., ionic aluminum Al(III) centers, for bonding to single-strand or double-strand DNA immobilized to said aluminum centers. The aluminum (III) alkanebisphosphonate can be provided as a biosensor having a coating of $Al_2(C_4BP)$ to bond to SS-DNA or ds-DNA.

A further object of the present invention is to provide a biosensor in the form of chips or electrodes with adsorbed DNA that is labelled with a luminescent label, such as an osmium or ruthenium moiety.

A still further object of the present invention is to prepare a biosensor by treating a silicon wafer to form a chromium layer and juxtaposed gold layer, then contacting the layered wafer with an anchoring agent; and subsequently immersing the product in $Al(NO_3)_3$, bisphosphonic acid ($H_2O_3P(CH_2)_4PO_3H_2$) and $Al(NO_3)_3$ aqueous solutions.

Another object of the present invention involves the detection of a nucleic acid by labelling with luminescent metal chelates.

A further object of the present invention is to apply electrogenerated chemiluminescent techniques to a plurality, i.e., arrayed, oligonucleotide probes.

These and other objects will become more apparent from the following detailed description and drawings.

Brief Description Of The Drawings

Fig. 1 shows a schematic representation of the silicon electrode of the present invention containing ionic aluminum Al(III) sites.

Fig. 2 shows immobilization of ds-DNA on a $Al_2(C_4BP)$ film and interaction of $Ru(phen)_3^{2+}$ with the ds-DNA.

Figs. 3A-3C show first (A), second (B), and third (C) scans. ECL emission-potential transients at the $Al_2(C_4BP)$/DNA-$Ru(phen)_3^{2+}$ electrode in 0.19 M phosphate buffer, pH 7, containing 0.13 M TPrA. (Scanning was halted and the solution stirred after each scan.) Scans were initiated at 0 V and were directed toward more positive potentials. Scan rate, 50 mV/s.

Figs. 4A-4C show schematic representations of immobilization of ss-DNA tagged with $Ru(bpy)_3^{2+}$ on the film (Fig. 4A); immobilization of ss-DNA on the film and hybridization of complementary strand DNA

tagged with $Ru(bpy)_3^{2+}$ (Fig. 4B); immobilization of poly(dA) on the film, hybridization of poly(dT), and then interaction of $Ru(phen)_3^{2+}$ with the ds-DNA(poly(dA)•poly(dT)) where the • represents an ECL active species.

Figures 5A-5C show a cyclic voltammogram (Fig. 5A); an emission-potential transient of the $Al_2(C_4BP)/\lambda$-1 ss-DNA-$Ru(bpy)_3^{2+}$ electrode in 0.19 M phosphate buffer/0.13 M TPrA, pH 7 (Fig. 5B); and an emission-potential transient of the $Al_2(C_4BP)\lambda$-1 ss-DNA in the same solution (Fig. 5C). The electrode used in the experiments shown in Figures 5A-5C was prepared as described in the specification with immersion in a 1.38 μM λ-1 ss-DNA-$Ru(bpy)_3^{2+}$ or λ-1 ss-DNA solution for ~4 h, respectively. In each case, the potential was scanned from 0 to 1.60 V at v = 50 mV/s.

Figure 6 shows an emission-time transient for the $Al_2(C_4BP)/\lambda$-1 ss-DNA-$Ru(bpy)_3^{2+}$ electrode in 0.19 M phosphate buffer/0.13 M TPrA, pH 7, when the potential was stepped from 0 to 1.5 V.

Figures 7A and 7B show ECL emission-potential transients at the $Al_2(C_4BP)/\lambda$-1c ss-DNA/λ-1

ss-DNA-$Ru(bpy)_3^{2+}$ electrode (Fig. 7A) and at the $Al_2(C_4BP)$/λ-1 ss-DNA/λ-1 ss-DNA-$Ru(bpy)_3^{2+}$ electrode (Fig. 7B) where both electrodes are immersed in 0.19 M phosphate buffer, pH 7, containing 0.13 M TPrA and the potential was scanned from 0 to 1.60 at scan rate, 50 Mv/s.

Figures 8A and 8B show ECL emission-potential transients at the $Al_2(C_4BP)$/poly(dA)/poly(dT)/$Ru(phen)_3^{2+}$ electrode (Fig. 8A) and at the $Al_2(C_4BP)$/poly(dA)/$Ru(phen)_3^{2+}$ electrode (Fig. 8B) where both electrodes are immersed in 0.19 M phosphate buffer, pH 7, containing 0.13 M TPrA, and the potential was scanned from 0 to 1.60 at scan rate, 50 mV/s.

Figures 9A-9C show TEM images of Au substrate coated on a Formvar film on a #400 Cu grid (Fig. 9A); the $Al_2(C_4BP)$ film on the Au substrate (Fig. 9B); immobilized calf thymus ds-DNA on the $Al_2(C_4BP)$ film, prepared by immersing the film in a 1.65 mM [NP] of ds-DNA solution for ~ 4 h (Fig. 9C).

Figures 10A-10C show TEM images of the $Al_2(C_4BP)$ film on Au substrate coated on a Formvar film (Fig. 10A); immobilized calf thymus

ds-DNA on the $Al_2(C_4BP)$ film, prepared by immersing the film in a 1.65 mM [NP] of ds-DNA solution, for ~4 h (Fig. 10B); immobilized sonicated calf thymus ds-DNA on the $Al_2(C_4BP)$ film, prepared by immersing the film in a 1.65 mM [NP] of ds-DNA solution, sonicated for ~6 h at room-temperature, for ~4 h. (Fig. 10C)

Figures 11A - 11B show sequencing via array hybridization.

Detailed Description Of Invention

The present invention relates to a sensor and method of detecting nucleic acids using the sensor. The sensor can be a chip, electrode, or an appropriately modified surface for adsorbing ss-DNA or ds-DNA. The nucleic acids detected by the method of the present invention include DNA, cDNA or any synthetic variant thereof. A nucleic acid as used throughout the specification and in the claims is meant DNA or any synthetic variant thereof.

Examples of DNAs detectable by the present method include chromosome DNA, plasmid DNA, viral DNA, bacterial DNA and recombinant DNA. The length of nucleic acid sequence capable of detection by the present method ranges from about 2.7 nm to about 200 nm. In a preferred embodiment, the nucleic acid sequence ranges from 8 base pair (bp) nucleotides to 3,000 base pair nucleotides. In a most

preferred embodiment ranges from about 30 bp nucleotides to 1,500 bp nucleotides.

In the present invention, the nucleic acid sequence to be detected may be of purified nucleic acid or may be present in a biological sample. Biological samples in which nucleic acids can be detected using the method of the present invention include but are not limited to biological fluids, e.g., serum, saliva, hair, skin, etc. Alternatively, the nucleic acid can be purified from a sample using methods known to those skilled in the art (Current Protocols in Electrobiology (1994 ed. Ausubel, F.M. et al. John Wiley & Sons, Inc.)).

The aluminum (III) alkanebisphosphonate preferably used is a $Al_2(C_4BP)$, also $[Al_2C_4BP]$, film bearing biosensor and is prepared as follows. Silicon wafers were soaked in trichloroethylene for 30 min, rinsed twice with 2-propanol, rinsed with excess amount of deionized water, and then dried with a stream of dry nitrogen. The clean silicon wafers were primed with a 50 Å chromium layer followed by deposition of a 2000 Å gold layer. Chromium and gold targets (99.999%) were used to sputter the films onto the wafers in a MRC Model 8620 system at 10^{-2} torr. Other techniques, such as chemical vapor deposition (CVD) to apply the Au or Cr layers, can also be used.

The gold surface supported on the silicon wafers was cleaned with hot chromic acid (saturated $K_2Cr_2O_7$ in 90% H_2SO_4) for ~10 s and then rinsed with copious amounts of water. This

process was repeated until the surface contact angle with water was less than 15°. The clean Au surface was then immediately soaked in an anchoring agent, 0.5 mM 4-mercaptobutylphosphonic acid (MBPA) solution in absolute ethanol for ~24 h. See Fig. 1. The phosphoric acid terminated surface was then thoroughly rinsed with the ethanol, dried with a stream of N_2 and then immersed alternately in 5 mM $Al(NO_3)_3$, 5 mM bisphosphonic acid ($H_2O_3P(CH_2)_4PO_3H_2$) and 5 mM $Al(NO_3)_3$ aqueous solution, taking ~4 h for each immersion, with washing with water between each step. Although the preferred embodiment contemplates the use of Al centers, other metal centers, such as, lanthanum (La) and zirconium (Zr) are also contemplated. In addition, although a C_4 specie was utilized in producing the alkanebisphosphonate film, other chain links, C_2-C_{16} may be utilized, which result in adequate spacing of the aluminum ions on the surface of the film to permit contact with the phosphates of the DNA backbone. In other words, the "spacer" may range from 2 to 16 carbons in length.

The sensor described above is used by:

a) adsorbing a nucleic acid onto a film containing metal centers;

b) reacting nucleic acid adsorbed to said film with a luminescent metal label; and

c) detecting the nucleic acid metal label chelates formed in step b) via electrogenerated chemiluminescence of said chelates.

In step (a), the film to which the nucleic acid is adsorbed should contain metal ions which are suitably spaced on the surface of the film to allow interaction of the metal with the phosphate backbone of the nucleic acid sequence. Examples of metal centers suitable for use in binding to nucleic acid phosphate groups are aluminum, lanthanum, and zirconium. In a preferred embodiment, the film contains an aluminum Al (III) metal center.

The nucleic acid adsorbed to the film in step (a) of the method of the present invention may be either double-stranded or single-stranded. When a single-stranded nucleic acid is adsorbed to the film, the adsorbed single-stranded nucleic acid is then hybridized to a complementary single-stranded nucleic acid sequence. Conditions of hybridization are utilized which promote base pairing between the single-stranded DNA adsorbed to the film in its complementary sequence. Factors influencing hybridization between nucleic acid sequences are known to those skilled in the art and include salt concentration of the hybridization solution, hybridization temperature and stringency of post-hybridization washes. In addition, the length of hybridization may also be controlled to optimize binding. Satiable buffers in which to carry out the hybridization reaction include 5mM Tris buffer, pH 7 containing 50 mM NaCl.

The complementary single-stranded nucleic acid sequence hybridized with the nucleic acid adsorbed to the film may be unlabeled or labeled with a luminescent metal label.

Suitable luminescent labels include ruthenium- and osmium-containing labels where ruthenium or osmium are bound to at least one polydentate ligand. If the metal has greater than one polydentate ligand, the polydentate ligands may be the same or different. (Other known ECL active labels can also be utilized that emit at different wave lengths such as organic ECL labels, e.g. sulfonated-9,10-diphenylanthracene.) Polydentate ligands of either ruthenium or osmium include aromatic and aliphatic ligands. Suitable aromatic polydentate ligands include aromatic heterocyclic ligands. Preferred aromatic heterocyclic ligands are nitrogen-containing, such as, for example, bipyridyl, bipyrazyl, terpyridyl, and phenanthrolyl. If the metal chelate has greater than one polydentate ligand, the polydentate ligands may be the same or different.

Suitable polydentate ligands may be unsubstituted, or substituted by any of a large number of substituents known to the art. Suitable substituents include for example, alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, substituted aralkyl, carboxylate, carboxaldehyde, carboxamide, cyano, amino, hydroxy, imino, hydroxycarbonyl, aminocarbonyl, amidine, guanidinium, ureide, sulfur-containing groups, phosphorous containing groups, and the carboxylate ester of N-hydroxysuccinimide.

The ruthenium or osmium may have one or more monodentate ligands, a wide variety of which are known to the art. Suitable monodentate ligands include, for example,

carbon monoxide, cyanides, isocyanides, halides, and aliphatic, aromatic and heterocyclic phosphines, amines, stibines, and arsines. A more complete list of the ligands, e.g., monodentate and polydentate ligands, that can be used in the present invention are set forth in U.S. Patent Nos. 5,310,687, 5,238,808 and 5,221,605, the subject matter of which are incorporated herein by reference.

It is also within the scope of this invention for one or more of the ligands of the metal to be attached to additional chemical labels, such as, for example, radioactive isotopes, fluorescent compounds, or additional luminescent ruthenium- or osmium-containing centers.

The complementary single-stranded nucleic acid may be tagged with the preferred luminescent metal labels of the present invention via co-valent bonding to one or more of the polydentate ligands of the metal label through one more amide linkages. This linkage may be oriented so that the nucleic acid is bonded directly either to the carbinol or to the nitrogen of the amide linkage. These chemical moieties may be ionized. A more elaborate description of methods for attaching a luminescent ruthenium-containing or osmium-containing label to amino groups of biological substances such as nucleic acid is provided in U.S. Patent No. 5,221,605 incorporated by reference.

In an alternative embodiment, the complementary single-stranded nucleic acid is unlabeled and hence, hybridization to a single-stranded nucleic acid adsorbed to a

film results in generation of unlabeled double-stranded nucleic acid adsorbed to the film. Thus, double-stranded nucleic acid can be adsorbed to the film directly or can be created by first adsorbing single-stranded nucleic acid to the film and then hybridizing the adsorbed nucleic acid with its complementary sequence. In either case, the film containing the adsorbed double-stranded nucleic acid is then immersed in a solution containing luminescent metal label or a solution suitable for promoting intercalation of the metal with the double-stranded nucleic acid. Examples of suitable solutions include, but are not limited to water.

A nucleic acid in which the luminescent metal containing labels intercalates to produce nucleic acid--metal label chelates is then detected by inducing the metal label present in the chelates to emit electromagnetic radiation by creating an excited state of the metal species that will luminesce at wave lengths from about 200 nanometers to about 900 nanometers, at ambient temperatures. Intercalation (or more generally, association) of the ECL labeled species with DNA depends upon the experimental conditions in which the label is partially inserted between the base pairs of DNA. It is considered "association" because of an electrostatic interaction between a positively-charged label and the negatively-charged phosphate groups on the DNA. The exact nature of the interaction of $Ru(phen)_3^{2+}$ with DNA is uncertain, but is believed to be intercalation. The temperature must be below the melting point of ds-DNA, preferably about 25-30° C.

The pH is typically near 7, but within a range of about 5 to about 8. The intercalation or association reaction must be given sufficient time to occur; about 30 to about 60 minutes, although times as short as 10 minutes also work.

In one embodiment of the present invention, the metal label is excited by exposing the nucleic acid-metal label chelates to electrochemical energy. The potential at which the oxidation of the metal label will occur depends upon the exact structure of the metal label as well as factors such as the co-reactant utilized, the pH of the solution and the nature of the electrode used. Examples of suitable co-reactants which, when incubated with the nucleic acid-metal label chelates in the presence of the electrochemical energy, will result in emission of the metal label intercalated with the nucleic acid, include tripropylamine (TPrA), oxalate or other organic acid such as pyruvate, lactate, malonate, tartrate and citrate. This oxidation can also be performed chemically, with some strong oxidants such as PbO_2 or a Ce(IV) salt.

Those of ordinary skill in the art recognize how to determine the optimal potential and emission wave length of an electrochemiluminescent system. The electrochemiluminescent species may be measured by any suitable mechanism such as measurement of an electric current or emitted electromagnetic radiation. For example, the rate of energy inputted into the system can provide a measure of the luminescent species. Suitable measurements include, for example, measurements of

electric current when the luminescent species is generated electrochemically, the rate of reductant or oxidant utilization when the luminescent species is generated chemically or the absorption of electromagnetic energy in photoluminescent techniques. In addition, of course, the luminescent species can be detected by measuring the emitted electromagnetic radiation. All of these measurements can be made either as continuous, rate-based measurements, or as cumulative methods which accumulate the signal over a long period of time. An example of rate-based measurements would be by using photomultiplier tubes, photodiodes or phototransistors to produce electric currents proportional in magnitude to the incident light intensity. Examples of cumulative methods are the integration of rate-based data, and the use of photographic film to provide cumulative data directly.

All of these luminescence-based methods entail repeated luminescence by the ruthenium-containing compound. The repetitive nature of the detectable event distinguishes these labels from radioactive isotopes or bound chemiluminescent molecules such as luminol. The latter labels produce a detectable event only once per molecule (or atom) of label, thereby limiting their detectability.

The following examples illustrate various aspects of the invention but are in no way intended to limit the scope thereof. Analysis was performed in a Plexiglas cell designed for ECL and electrochemical studies using the film of the

present invention, a gold surface supported on the silicon wafer containing metallic sites, e.g., Al. A saturated calomel reference electrode (SCE) and a platinum wire counter electrode were used for all measurements. The electrochemical measurements coupled with ECL experiments were carried out with a Model 175 universal programmer, a Model 173 potentiostat (Princeton Applied Research, PAR, Princeton, NJ), and an Ominigraphic 2000 X-Y recorder (Houston Instruments, Houston, TX). The ECL emission was detected by a Model C123 single-photon-counting system (Hamamatsu Corp., Bridgewater, NJ) utilizing a Hamamatsu R928P PMT, cooled to -20°C in a Model TE 308 TSRF cooler controller (Products for Research Inc., Danvers, MA). The meter output was fed into the y-axis of the x-y recorder, and the signal from the potentiostat was fed into the x-axis to afford ECL intensity versus bias potential display. Solution analysis by ECL was carried out with a QPCR analyzer (Perkin-Elmer, Norwalk, CT).

A MRC (Materials Research Corporation, Orangeburg, NY) Model 8620 sputtering system at 10^{-2} torr, with an RF power of 150 W and RF peak to peak voltage of 1.8 KV, was used to sputter gold (99.999%) on silicon wafers.

Polydeoxyadenylic acid (Poly(dA)), polythymidylic acid (poly(dT), polydeoxycytidylic acid (poly(dC)) and calf thymus (CT) ss-DNA and ds-DNA were obtained from Sigma Chemical Co. (St. Louis, MO) and were used without additional purification. The ss-DNA samples, λ-1 DNA (5'GAAAATGTGCTGACCGGACATGAAAATGAG3'), (Seq. ID. No. 1) λ DNA

tagged with $Ru(bpy)_3^{2+}$(5'$Ru(bpy)_3^{2+}$-GAAAATGTGCTGACCGGACATGAAAATGAG3'), (Seq. ID. No. 2) and a complementary strand λ-1c DNA (5'CTCATTTTCATGTCCGGTCAGCACATTTTC3'), were obtained from Perkin-Elmer and diluted with a 5 mM tris buffer containing 50 mM NaCl(pH 7). Synthesis of ss-DNA can be carried out on a DNA synthesizer (e.g. Applied Biosystems, Model 381A). See also L.J. McBride and M.H. Cruthers, Tetrahedron Letters, 24, 245 (1983).

The reagents used in the following examples include trichloroethylene (99.6%), 2-propanol (99.9%), tripropylamine (TPrA) (98%), $Ru(bpy)_3Cl_2.6H_2O$, $Ru(phen)_3Cl_2O$, ethyl alcohol (200 proof), $Al(NO_3)_3.9H_2O$, $K_2Cr_2O_7$, NaH_2PO_4 and tris(hydroxymethyl) aminomethane and were used as received without purification. Bisphosphonic acid $H_2O_3P(CH_2)_4PO_3H_2$ (C_4BPA), and 4-mercaptobutylphosphonic acid (MBPA) were synthesized in accordance with the techniques taught by Mallouk et al, J.A.C.S., 115, 11855 (1993). The assay buffer for ECL experiments contained 0.13 M TPrA and 0.19 M phosphate buffer, prepared by dissolving TPrA into a NaH_2PO_4 solution and adjusting the pH to 7 with 1 M NaOH.

Deionized water from a Millipore Milli-Q (18 MΩ-cm) system was used to prepare all aqueous solutions and to rinse the electrode surface.

TEM Sample Preparation.

TEM samples were prepared by coating Au on a Formvar film on a #400 Cu grid with a vacum evaporator (Edwards 306),

fabricating the $Al_2(C_4BP)$ film on Au following the procedure described above and then immobilizing DNA on the $Al_2(C_4BP)$ film. A transmission electron microscope (JEOL 100CX) at 80 KV was used to image the Au substrate, the $Al_2(C_4BP)$ film and the immobilized DNA.

Example 1

Electrogenerated Chemiluminescent Detection of Immobilized ds-DNA using A Ruthenium Label.

Calf thymus ds-DNA was immobilized on the surface of an $Al_2(C_4BP)$ film by immersing the film in a solution of DNA (1.9 mM in nucleotide phosphate, NP) for 4 h (Figs. 1 and 2). The film was then rinsed three times with 4-mL portions of deionized water and then immersed for 4 h in either an aqueous 0.56 mM $Ru(phen)_3Cl_2$ solution or a 0.12 mM $Ru(phen)_3(ClO_4)_2$ solution in MeCN. $Ru(phen)_3^{2+}$ associates with ds-DNA and can be detected through its electrogenerated chemiluminescence (ECL). Alternatively, the film could be soaked in a mixed ds-DNA (1.9 mM NP) and $Ru(phen)_3Cl_2$ (0.12mM) solution for 4 h to produce the adsorbed layer.

ECL was produced by scanning the potential of the electrode following film formation, DNA adsorption, and $Ru(phen)_3^{2+}$ association, from 0 to 1.6 V vs. a saturated calomel electrode (SCE) while it was immersed in a solution of 0.19 M phosphate buffer (pH 7) containing 0.13 M tri-n-propylamine (TPrA). Typical ECL transients, detected with a single-photon-counting apparatus, are shown in Figures 3A-3C. Emission arises from the energetic electron-transfer reaction between electrogenerated $Ru(phen)_3^{3+}$ and an intermediate in the oxidation of TPrA:

$$DNA\text{-}Ru(phen)_3^{2+}\ (ads) - e \rightarrow DNA\text{-}Ru(phen)_3^{3+} \quad [1]$$

$$CH_3CH_2CH_2\text{-}\ddot{N}\text{-}Pr_2 - e \rightarrow CH_3CH_2CH_2\dot{N}Pr_2^{+} \quad [2]$$

$$CH_3CH_2CH_2\dot{N}Pr_2^{+} \rightarrow CH_3CH_2\dot{C}HNPr_2 + H^{+} \quad [3]$$

$$DNA\text{-}Ru(phen)_3^{3+} + CH_3CH_2\dot{C}HNPr_2 \rightarrow DNA\text{-}Ru(phen)_3^{2+*} + CH_3CH_2CHNPr_2^{+} \quad [4]$$

$$DNA\text{-}Ru(phen)_3^{2+*} \rightarrow DNA\text{-}Ru(phen)_3^{2+} + h\upsilon \quad [5]$$

The emission intensity decreased on a second scan and none was seen on a third, suggesting loss of $Ru(phen)_3^{3+}$ from the film and diffusion into the bulk solution.

In a control experiment, an electrode with a film of $Al_2(C_4BP)$ that had not been treated with DNA was soaked for 4 h in either an aqueous 0.56 mM $Ru(phen)_3^{2+}$ or a 0.12 mM $Ru(phen)_3^{2+}$ in MeCN solution and then rinsed with MeCN and water. This electrode showed no ECL emission upon potential sweep in the same TPrA solution described above, demonstrating the adsorption of $Ru(phen)_3^{2+}$ on the $Al_2(C_4BP)$ film does not occur. The ability to generate $Ru(phen)_3^{3+}$ electrochemically in this ECL experiment demonstrates that the $Al_2(C_4BP)$ film and the DNA layer do not prevent heterogeneous electron-transfer reactions.

To obtain further evidence for $Al_2(C_4BP)$ film formation and DNA immobilization, experiments were undertaken with a gold-coated quartz crystal and a quartz crystal microbalance (QCM). The gold was repeatedly treated with hot chromic acid and then rinsed with water and EtOH until the surface was hydrophilic, as indicated by contact angle measurements. The crystal frequency was then measured in air during different stages of $Al_2(C_4BP)$ film formation, after DNA adsorption, and after $Ru(phen)_3^{2+}$ association. The film was rinsed after each step with deionized water and dried in a

stream of N_2 before measurement of the frequency. Results are shown in Table 1.

Table 1. Frequency and Mass Changes on a QCM Plate for Film Growth, DNA Immobilization, and $Ru(phen)_3^{2+}$ Binding.

After immersion in: [a]	Δf[g] (Hz)	Δm (ng)	$10^9\Gamma$[h] (mol/cm^2)
MBPA[b]	-70	137	1
$Al(NO_3)_3$[c]	-27	53	1[i]
C_4BPA[d]	-145	283	2
$Al(NO_3)_3$[c]	-25	49	1[i]
ds-DNA[e]	-24	47	0.3[j]
$Ru(phen)_3^{2+}$[f]	-18	35	0.1

[a] Immersions were sequential in order given from top to bottom; bare surface was gold; bare crystal frequency, 6.011329 MHz.

[b] 0.5 mM MBPA in EtOH for ~ 24 h.

[c] 5 mM aq. $Al(NO_3)_3$ for ~ 4 h.

[d] 5 mM phosphoric acid for 4 h.

[e] Calf thymus DNA (3.8 mM NP) for 4 h.

[f] 0.24 mM $Ru(phen)_3^{2+}$ in MeCN for 4 h.

[g] The variation and drift of the signal over the series of measurements was about $\pm$ 1 Hz.

[h] Assuming roughness factor for gold of 2 (total surface area, 0.6 cm^2). For comparison, for a close-packed monolayer of MBPA, $\Gamma = 0.6 \times 10^{-9}$ mol/cm^2.

[i] Assuming an $Al(H_2O)_3^{3+}$ until adsorbed.

[j] Corresponding to moles of nucleotide phosphate (NP) per cm^2.

Clearly, the crystal frequency decreases as the $Al_2(C_4BP)$ film forms and DNA and $Ru(phen)_3^{2+}$ are adsorbed on the surface, showing an increase of mass on the crystal during the different stages. The mass change, Δm, can be related to the

frequency change, Δf, by the Sauerbrey equation:

$$\Delta m = -(A\sqrt{\rho_q \mu_q}/2F_0^2)\Delta f$$

where F_0 is the fundamental frequency of the unloaded crystal (6 MHz), A is the electrode area (0.159 cm^2), ρ_q is the density of quartz (2.65 g/cm^3) and μ_q is the shear modulus of quartz (2.95 x 10^{11} $dyne/cm^2$). With these constants,

$$\Delta m \text{ (ng)} = -1.95\ \Delta f \text{ (Hz)}$$

The mass changes calculated in this way are also given in Table 1. These can be converted to approximate surface concentrations, Γ, assuming a roughness factor of 2 (i.e., total surface area of both sides of the quartz crystal of 0.6 cm^2). ECL could also be detected from both gold surfaces of the quartz crystal, when used as a substrate for $Al_2(C_4BP)$ film formation, DNA adsorption and $Ru(phen)_3^{2+}$ association and then scanned in the TPrA solution.

The electrode surface can be designed with immobilized DNA without adsorbing a detector molecule, $Ru(phen)_3^{2+}$. A ds-DNA on the surface can be detected by electrogenerated chemiluminescence of associated $Ru(phen)_3^{2+}$. Single-stranded DNA can also be immobilized on the $Al_2(C_4BP)$ film surface and then hybridized with complementary DNA in solution with detection of the ds-DNA produced by ECL.

Example 2

Single-strand DNA immobilization and hybridization.

λ-1 tagged ss-DNA (i.e., labeled with $Ru(bpy)_3^{2+}$) was immobilized on an aluminum phosphate film of the present invention by immersing the film in the DNA solution (Fig. 4A). The amount of immobilized DNA-$Ru(bpy)_3^{2+}$ on the surface was determined by ECL resulting from the oxidation of $Ru(bpy)_3^{2+}$ and TPrA in a solution.

Untagged λ-1c ss-DNA was immobilized on an aluminum phosphate of the present invention. The λ-1c ss-DNA containing film was incubated in a complementary strand λ-1 tagged ss-DNA solution at 60°C for 5 min and then cooled to room temperature gradually; during this cooling the ss-DNA hybridized with the complementary strand DNA (Fig. 4B). The hybridized DNA-$Ru(bpy)_3^{2+}$ on the film was detected by ECL as described above.

Poly(dA) was immobilized on an aluminum phosphate film of the present invention by soaking the film in a poly(dA) solution. After the immobilization, poly(dT) was hybridized with poly(dA) to produce poly(dA)•poly(dT) ds-DNA on the surface by incubating the film in a poly(dT) solution at 70°C for 5 min and then cooled to the room temperature gradually (Fig. 4C). To intercalate $Ru(phen)_3^{2+}$ into the ds-DNA (poly(dA)•poly(dT)), the $Al_2(C_4BP)$/poly(dA)•poly(dT) film was treated with a $Ru(phen)_3^{2+}$ solution. The hybridized poly(dA)•poly(dT)-$Ru(phen)_3^{2+}$ on the surface was determined by ECL based on the oxidation of $Ru(phen)_3^{2+}$ and TPrA in the

solution.

Example 3

Immobilization and Detection of 30 bp ss-DNA Immobilized On Aluminum Phosphate Film.

The aluminum phosphate film, prepared as described above was immersed in a 1.38 μM solution of λ-1 30 bp ss-DNA (tagged with $Ru(bpy)_3^{2+}$) for ~2 h. This was employed as a working electrode for an ECL experiment in a 0.19 M phosphate buffer (pH 7) containing 0.13 M TPrA. Cyclic voltammograms and emission transients were obtained by scanning the potential of the electrode from 0 to 1.6 V vs, a saturated calomel electrode (SCE). A representative voltammogram and emission detected with a single-photon-counting system are shown in Figures 5A-5C. The broad oxidation was at ~ 1.2 V and a small reduction wave at ~0.35 V observed in the voltammogram (Fig. 5A) arise from the oxidation of TPrA, $Ru(bpy)_3^{2+}$ and the Au substrate and the reduction of the oxide of Au. The ECL emission from the $Al_2(C_4BP)$/λ-1 ss-DNA-$Ru(bpy)_3^{2+}$ electrode (Fig. 5B) demonstrates that the λ-1 30 bp ss-DNA-$Ru(bpy)_3^{2+}$ was immobilized on the film. The decay of the light intensity (I) as a function of time (t) was also investigated with the single-photon-counting system. The light intensity detected by the photomultiplier tube, when the potential was stepped from 0 to 1.50 V, decreased with time (Fig. 6). The decay of intensity with time suggests desorption of ss-DNA-$Ru(bpy)_3^{2+}$ from the electrode surface or

irreversible decomposition of the emitter. Emission results from the energetic electron-transfer reaction between electrogenerated $Ru(bpy)_3^{3+}$ and an intermediate in the oxidation of TPrA:

$$DNA\text{-}Ru(bpy)_3^{2+}\ (ads) \rightarrow DNA\text{-}Ru(bpy)_3^{3+} + e^- \quad [6]$$

$$CH_3CH_2CH_2\dot{N}Pr_2 - e \rightarrow CH_3CH_2CH_2\dot{N}Pr_2^+ \quad [7]$$

$$CH_3CH_2CH_2\dot{N}Pr_2^+ \rightarrow CH_3CH_2\dot{C}HNPr_2 + H^+ \quad [8]$$

$$DNA\text{-}Ru(bpy)_3^{3+} + CH_3CH_2\dot{C}HNPr_2 \rightarrow DNA\text{-}Ru(bpy)_3^{2+*} + CH_3CH_2CHNPr_2^+ \quad [9]$$

$$DNA\text{-}Ru(bpy)_3^{2+*} \rightarrow DNA\text{-}Ru(bpy)_3^{2+} + h\upsilon \quad [10]$$

No ECL emission was observed from a film prepared by immersing it in solution of unlabeled ss-DNA: 1.38 μM λ-1 30 bp ss-DNA or a 0.37 μM λ-1c 30 bp ss-DNA solution (Fig. 5C).

A control experiment with the aluminum phosphate film immersed in a 304 nM $Ru(bpy)_3^{2+}$ solution for ~ 4 h shows a negligible ECL signal, indicating that $Ru(bpy)_3^{2+}$ does not adsorb on the film and oxidize.

Example 4

Detection of 30 bp ss-DNA Hybridized to Complementary SS-DNA Tagged With A Ruthenium Label.

After the immobilization of unlabeled λ-1c 30 bp ss-DNA on the $Al_2(C_4BP)$ film, the film was immersed in a 1.38 μM complementary strand ss-DNA (tagged with $Ru(bpy)_3^{2+}$) (λ-1 30 bp ss-DNA-$Ru(bpy)_3^{2+}$) solution. The film in the solution was gradually heated to 60°C in water bath, incubated at 60°C for

5 min. and then slowly cooled to room-temperature, during which the λ-1 ss-DNA-$Ru(bpy)_3^{2+}$ was hybridized with the complementary strand λ-1c ss-DNA on the surface as illustrated in Fig. 4B. The film with hybridized DNA was employed as the working electrode in an ECL cell as described above. The ECL emission from the film was observed as shown in Fig. 7A. However, when λ-1 ss-DNA, rather than λ-1c ss-DNA, was immobilized on the film and followed by the same hybridization procedure as described above by incubating the film in the λ-1 ss-DNA-$Ru(bpy)_3^{2+}$ solution, heating to 60°C, and then cooling to room-temperature, no obvious ECL emission was evident as shown in Fig. 7B. This demonstrates that the ECL emission in Fig. 7A arises from the hybridization of immobilized λ-1c ss-DNA with λ-1 ss-DNA-$Ru(bpy)_3^{2+}$ in the solution. The emission again results from the oxidation of $Ru(bpy)_3^{2+}$-tagged hybridized ss-DNA and TPrA in the solution as described by Eqs. [6] - [10]. In this experiment the $Al_2(C_4BP)$ film should be incubated in the λ-1 ss-DNA solution for a sufficiently long time (at least 4 h) to cover all possible Al (III) binding sites on the film with the λ-1 ss-DNA before the $Al_2(C_4BP)$/λ-1 ss-DNA electrode is exposed to the λ-1 ss-DNA-$Ru(bpy)_3^{2+}$. It is preferred to cover all Al^{3+} adsorption sites to avoid emission from the film/λ-1 ss-DNA/λ-1 ss-DNA-$Ru(bpy)_3^{2+}$ arising from some immobilization of ss-DNA-$Ru(bpy)_3^{2+}$.

Example 5

Detection of poly(dA) Hybridized to poly(dT) using a Ruthenium Label.

The aluminum phosphonate film, prepared as described above, was immersed in a 21 μM poly(dA) solution for ~4 h, then in a 0.24 mM $Ru(phen)_3^{2+}$ solution for ~4 h. When the $Al_2(C_4BP)$/poly(dA)/$Ru(phen)_3^{2+}$ film was employed as a working electrode in a solution of 0.19 M phosphate buffer (pH 7) containing 0.13 M TPrA, no ECL emission was observed (Fig. 8A). This is consistent with the lack of association of $Ru(phen)_3^{2+}$ with ss-DNA. However, a film of poly(dA) hybridized with only poly(dT) to form ds-DNA did produce ECL. This film, formed by immersion in the poly(dA)solution, was incubated in a 38 μM poly(dT) solution (gradually heated to 70°C in a water bath, incubated at 70°C for 5 min and then slowly cooled to room temperature). It was then treated with a 0.24 mM $Ru(phen)_3^{2+}$ aqueous solution and used as a working electrode in the TPrA-containing phosphate buffer for ECL investigation. ECL emission was observed as shown in a representative emission transient (Fig. 8B), demonstrating that poly(dT) in the solution hybridized with the poly(dA) on the surface to produce a ds-DNA [poly(dA)•poly(dT] and $Ru(phen)_3^{2+}$ intercalated with the ds-DNA as shown in Fig. 4c.

These examples show that $Ru(phen)_3^{2+}$ only intercalates with ds-DNA [poly(dA)•poly(dT], but not with ss-DNA (poly(dA)). Intercalation of $Ru(phen)_3^{2+}$ with ss-DNA and ds-DNA, ECL emission was measured from a 43 nM $Ru(phen)_3^{2+}$

solution, a 43 nM $Ru(phen)_3^{2+}$ containing 57 μM nucleotide phosphate ([NP]) of calf thymus ss-DNA and a 43 nM $Ru(phen)_3^{2+}$ containing 33 μM [NP] of calf thymus ds-DNA with a QPCR analyzer. Results are given in Table 2,

Table 2

ECL of Solutions of 43 nM $Ru(phen)_3^{2+}$

with ss-DNA and ds-DNA

Solution	I $Ru(phen)_3^{2+}$ alone	II[a] ss-DNA+$Ru(phen)_3^{2+}$	III[b] ds-DNA+$Ru(phen)_3^{2+}$
Conc. NP(μM)	0	57	33
ECL(cts/s)	10068±23	10052±12	7779±268

[a] Ratio of moles of nucleotide phosphate (NP) of ss-DNA to $Ru(phen)_3^{2+}$ = 1.3×10^4

[b] Ratio of moles of nucleotide phosphate (NP) of ds-DNA to $Ru(phen)_3^{2+}$ = 7.7×10^3

indicating that the ECL emission decreased after ds-DNA was added to the $Ru(phen)_3^{2+}$ solution. A negligible change, however, in the ECL signal was shown when ss-DNA was added, further demonstrating that $Ru(phen)_3^{2+}$ only intercalates with ds-DNA, but not with ss-DNA.

A control experiment in which a film, after the immobilization of poly(dA), was incubated in a 21 μM poly(dA) or a 46 μM poly(dC) solution instead of poly(dT) (70°C for 5 min then cooled to room-temperature) and then treated with $Ru(phen)_3^{2+}$ for ~2 h produces no ECL emission. This indicates that the $Al_2(C_4BP)$/poly(dA) electrode can distinguish a complementary strand of poly(dT) DNA from non-complementary ones.

Example 6

TEM Images of the film and calf thymus ds-DNA.

Samples, prepared by coating Au on a Formvar film on a #400 Cu grid with a vacuum evaporator, fabricating the aluminum phosphate film on the Au as described above, and then immobilizing DNA on the $Al_2(C_4BP)$ film by immersing the film in a 1.65 mM [NP] of calf thymus ds-DNA for 4 h, were imaged with a transmission electron microscope (TEM) at 80 KV. As shown in Figs. 9A-9C the featureless Au substrate (Fig. 9A) shows formation of crystalline islands of $Al_2(C_4BP)$ film (Fig. 9B) and clumps of DNA (Fig. 9C). The film (Fig. 10A) was then treated with either 1.65 mM [NP] calf thymus ds-DNA (Fig. 10B) or an identical solution of ds-DNA that had been subjected to sonication for 6 h (Fig. 10C). The results indicate that smaller clumps of DNA are adsorbed on the film after sonication.

A further embodiment of the invention is shown in Figs. 11A and 11B. A sensor surface having a multilayer film with bonding groups is provided with a complete set of oligonucleotide probes using similar techniques described above for adsorbing ds-DNA and for SS-DNA. See Fig. 11A. The sensor surface of Fig. 11A is contacted with DNA to be sequenced. The above disclosed ECL procedures are then used to recognize zones with a complementary sequence. (Fig. 11B).

When chips are used, different types of ss-DNA that make up a test sequence are attached to the surface of a chip to make an array. The chip array is then exposed to the

sample solution to be sequenced, with formation of ds-DNA at the appropriate location being recognized by the ECL approach.

Although the invention has been described in conjunction with the specific embodiments, it is evident that many alternatives and variations will be apparent to those skilled in the art in light of the foregoing description. Accordingly, the invention is intended to embrace all of the alternatives and variations that fall within the spirit and scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Allen J. Bard and Xia-Hong Hu

(ii) TITLE OF THE INVENTION: BIOSENSOR FOR AND METHOD OF ELECTROGENERATED CHEMILUMINESCENT DETECTION OF NUCLEIC ACID ADSORBED TO A SOLID SURFACE

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Morgan & Finnegan
(B) STREET: 1299 Pennsylvania Ave., N.W., Suite 960
(C) CITY: Washington
(D) STATE: District of Columbia
(E) ZIP CODE: 20004

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 3.50 inch, 1.44 Mb storage
(B) COMPUTER: IBM COMPATIBLE
(C) OPERATING SYSTEM: MS-DOS 5.0
(D) SOFTWARE: WordPerfect 5.1

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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Michael S. Marcus
(B) REGISTRATION NUMBER: 31,727
(C) REFERENCE/DOCKET NUMBER: 23247001

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202) 857-7887
(B) TELEFAX: (202) 857-7929

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: LAMBDA

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME SEGMENT: N/A
(B) MAP POSITION: N/A
(C) UNITS: N/A

(ix) FEATURE:
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(B) LOCATION: N/A
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: N/A

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAAAATGTGC TGACCGGACA TGAAAATGAG 30

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30
(B) TYPE:
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: N/A

(v) FRAGMENT TYPE: LAMBDA

(vi) ORIGINAL SOURCE:
(A) ORGANISM:
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE:

(viii) POSITION IN GENOME:
(A) CHROMOSOME SEGMENT: N/A
(B) MAP POSITION: N/A
(C) UNITS: N/A

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(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES In SEQ ID NO: 2:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CTCATTTTCA TGTCCGGTCA GCACATTTTC 30

CLAIMS

1. A method of detecting nucleic acids comprising:
   a. forming a sensor surface bearing a film having metallic centers;
   b. adsorbing at least one nucleic acid onto to said metal centers;
   c. reacting nucleic acid adsorbed to said film with a luminescent metal label; and
   d. detecting the nucleic acid metal label chelates formed in step (c) via electrogenerated chemiluminescence of said chelates.

2. The method of claim 1, wherein the nucleic acid of step (b) is double-stranded.

3. The method of claim 1, wherein the nucleic acid of step (b) is single-stranded.

4. The method of claim 3, wherein step (c) further comprises binding the single-stranded nucleic acid adsorbed to the film, to a complementary single-stranded nucleic acid sequence.

5. The method of claim 1, wherein said electrogenerated chemiluminescence arises from a reaction of an electrogenerated metal label in the nucleic acid -metal label

chelate and a suitable coreactant.

6. The method of claim 5, wherein said electrogenerated metal label is produced via emission from the sensor which is an electrode.

7. A method of detecting nucleic acids comprising:

a. forming an electrode by adsorbing at least one nucleic acid onto said electrode;

b. reacting nucleic acid adsorbed to said electrode with a luminescent label; and

c. detecting the nucleic acid label formed in step (b) via electrogenerated chemiluminescence.

8. The method of claim 7, wherein the nucleic acid of step (b) is double-stranded.

9. The method of claim 7, wherein the nucleic acid of step (b) is single-stranded.

10. The method of claim 8, wherein step (c) further comprises binding the single-stranded nucleic acid adsorbed to the electrode, to a complementary single-stranded nucleic acid sequence.

11. The method of claim 7, wherein said electrogenerated chemiluminescence arises from a reaction of an

electrogenerated metal label in the nucleic acid - metal label chelate and a suitable coreactant.

12. The method of claim 11, wherein said electrogenerated metal label is produced via emission from the electrode.

13. An aluminum alkanebisphosphonate biosensor comprising:

a substrate and a first metallic layer; and

an aluminum alkanebisphosphonate layer forming (Al) metallic centers.

14. A biosensor according to claim 13, wherein said biosensor further includes single-strand DNA immobilized to said aluminum centers.

15. A biosensor according to claim 14, wherein said DNA is labelled with and an osmium or ruthenium moiety.

16. A biosensor according to claim 13, wherein said biosensor further includes double-strand DNA immobilized to said aluminum centers.

17. A biosensor according to claim 16, wherein said DNA is labelled with an osmium or ruthenium moiety.

18. A method of preparing a biosensor for nucleic acid, comprising:

a) treating a silicon wafer to form a chromium layer and juxtaposed gold layer;

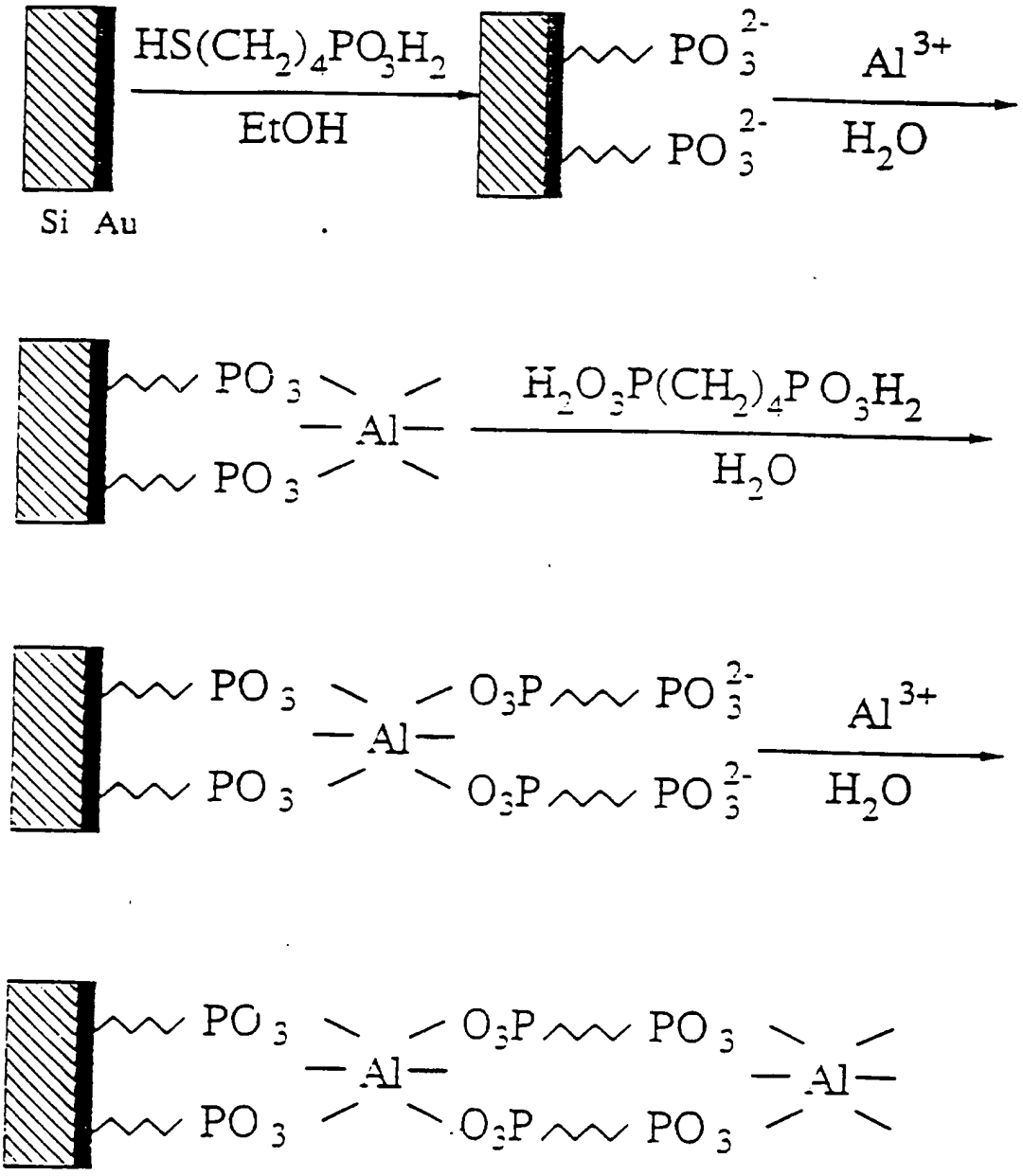
b) contacting said layered wafer with an anchoring agent; and

c) immersing the product of step b) alternately in $Al(NO_3)_3$, bisphosphonic ($H_2O_3P(CH_2)_4PO_3H_2$) and $Al(NO_3)_3$ aqueous solutions.

19. A biosensor comprising:

an electrode substrate, adsorbed ss-DNA or ds-DNA immobilized on said electrode, and a detectable label on said ss-DNA or ds-DNA.

20. A biosensor according to claim 19, wherein said DNA is labelled with an osmium or ruthenium moiety or a luminescent label.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PO3 2-
Al3+
H2O
PO3
Al
PO3
H2O3P(CH2)4PO3H2
H2O
PO3
Al
PO3
O3P
PO3 2-
O3P
PO3 2-
Al3+
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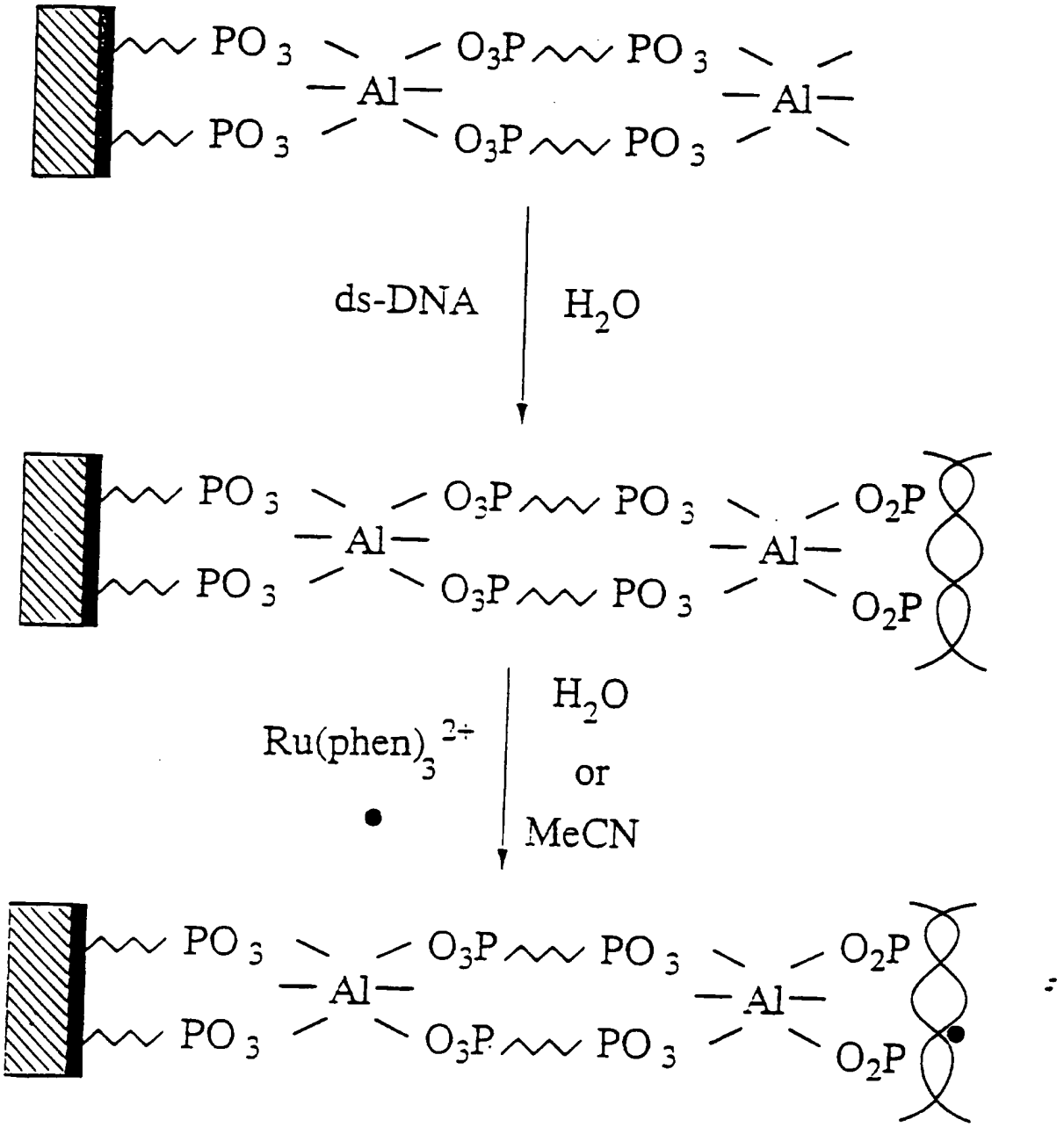
PO3
Al
PO3
O3P
PO3
PO3
Al
PO3
O3P
PO3

FIG. 1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O3P
PO 3
Al
Al
PO 3
O3P
PO 3
ds-DNA
H2O
PO 3
O3P
PO 3
O2P
Al
Al
PO 3
O3P
PO 3
O2P
Ru(phen)3 2+
H2O
or
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O3P
PO 3
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O3P
PO 3
O2P

FIG. 2

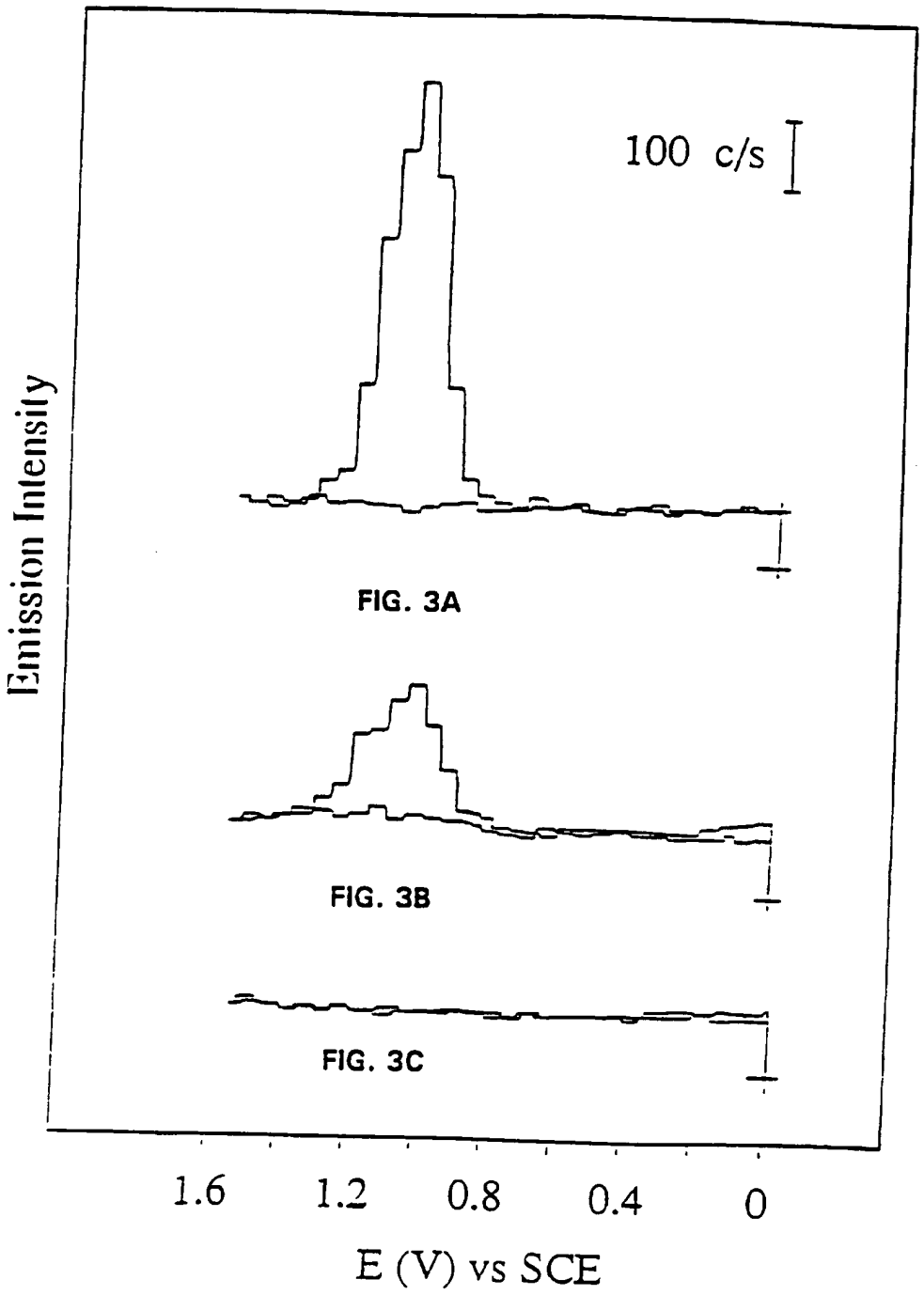
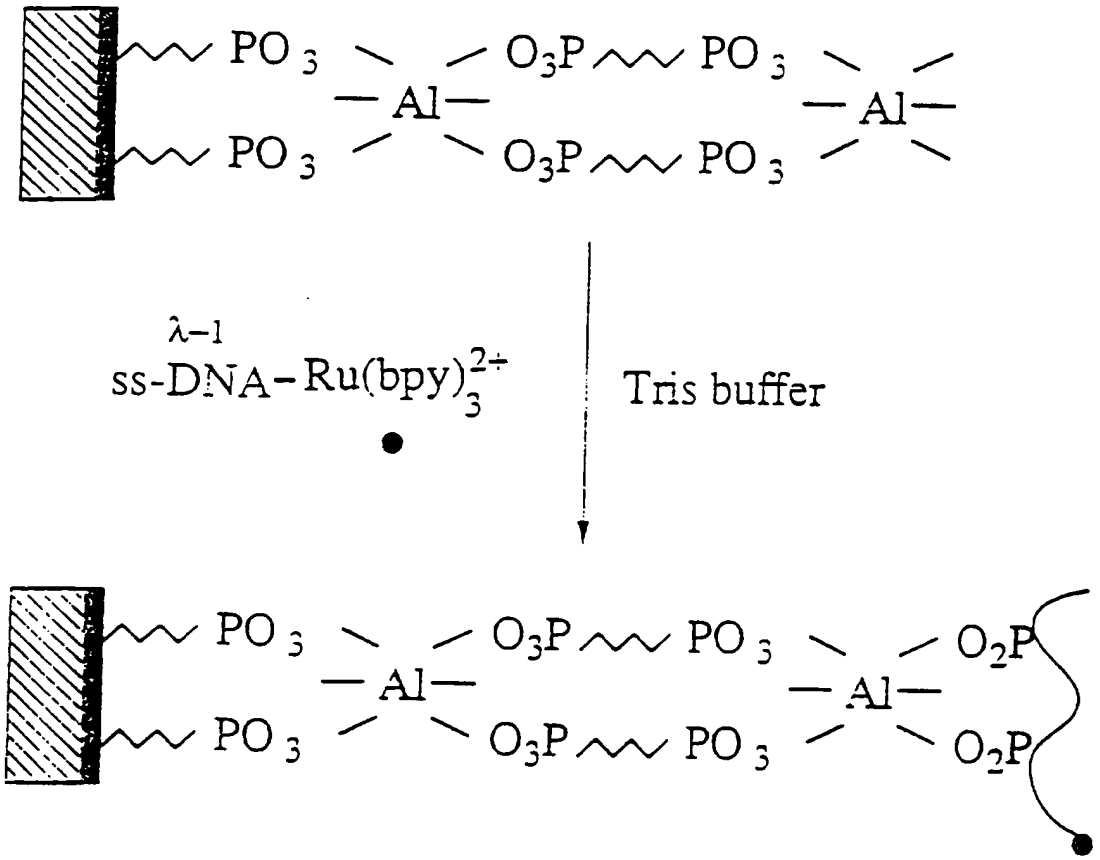


FIG. 3A

FIG. 3B

FIG. 3C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O3P
PO 3
Al
Al
PO 3
O3P
PO 3
λ-1
ss-DNA-Ru(bpy)3 2+
Tris buffer
PO 3
O3P
PO 3
O2P
Al
Al
PO 3
O3P
PO 3
O2P

FIG. 4A

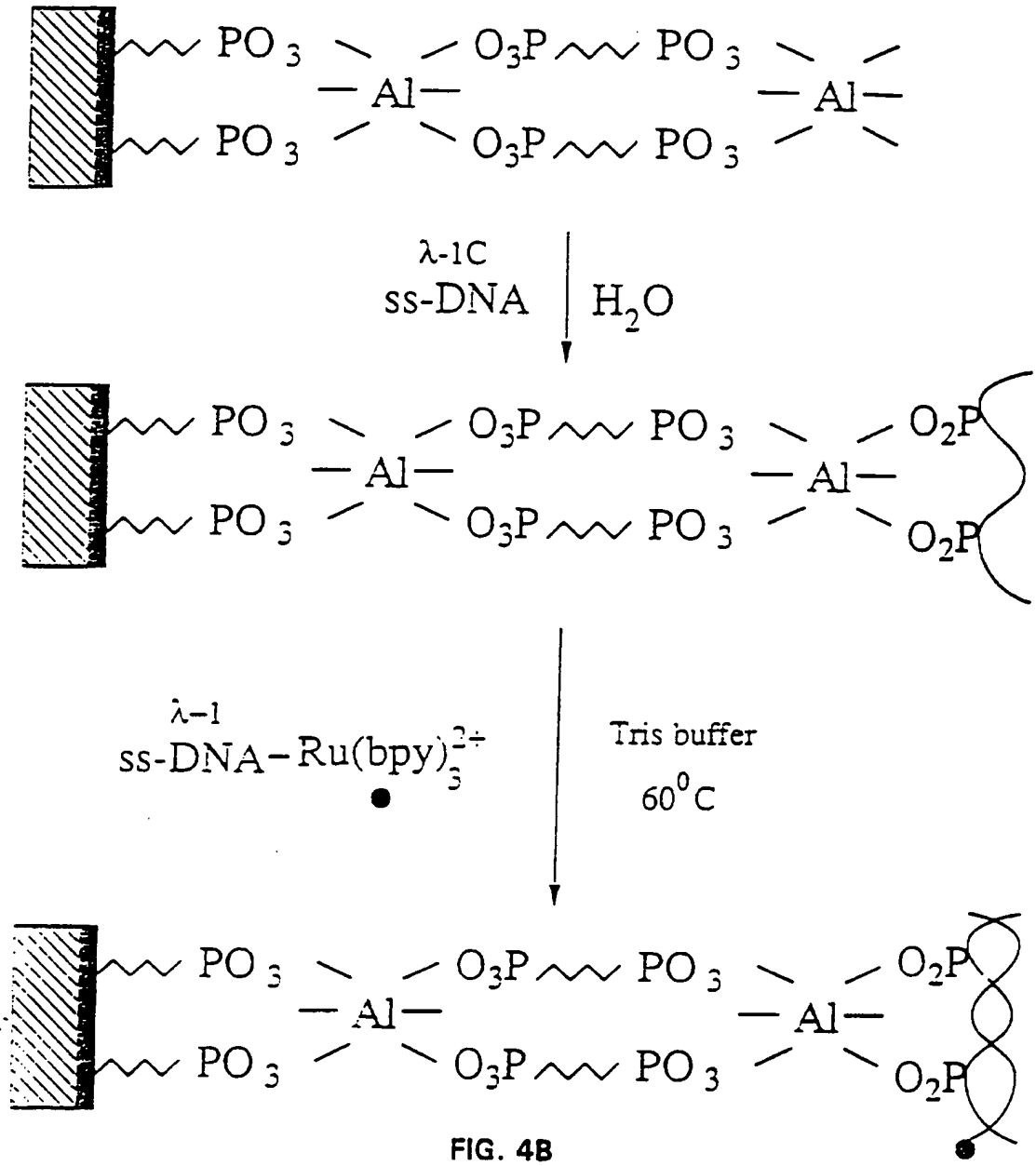

PO 3
O3P
PO 3
Al
Al
PO 3
O3P
PO 3
λ-1C
ss-DNA
H2O
O2P
O2P
λ-1
ss-DNA-Ru(bpy)3 2+
Tris buffer
60°C

FIG. 4B

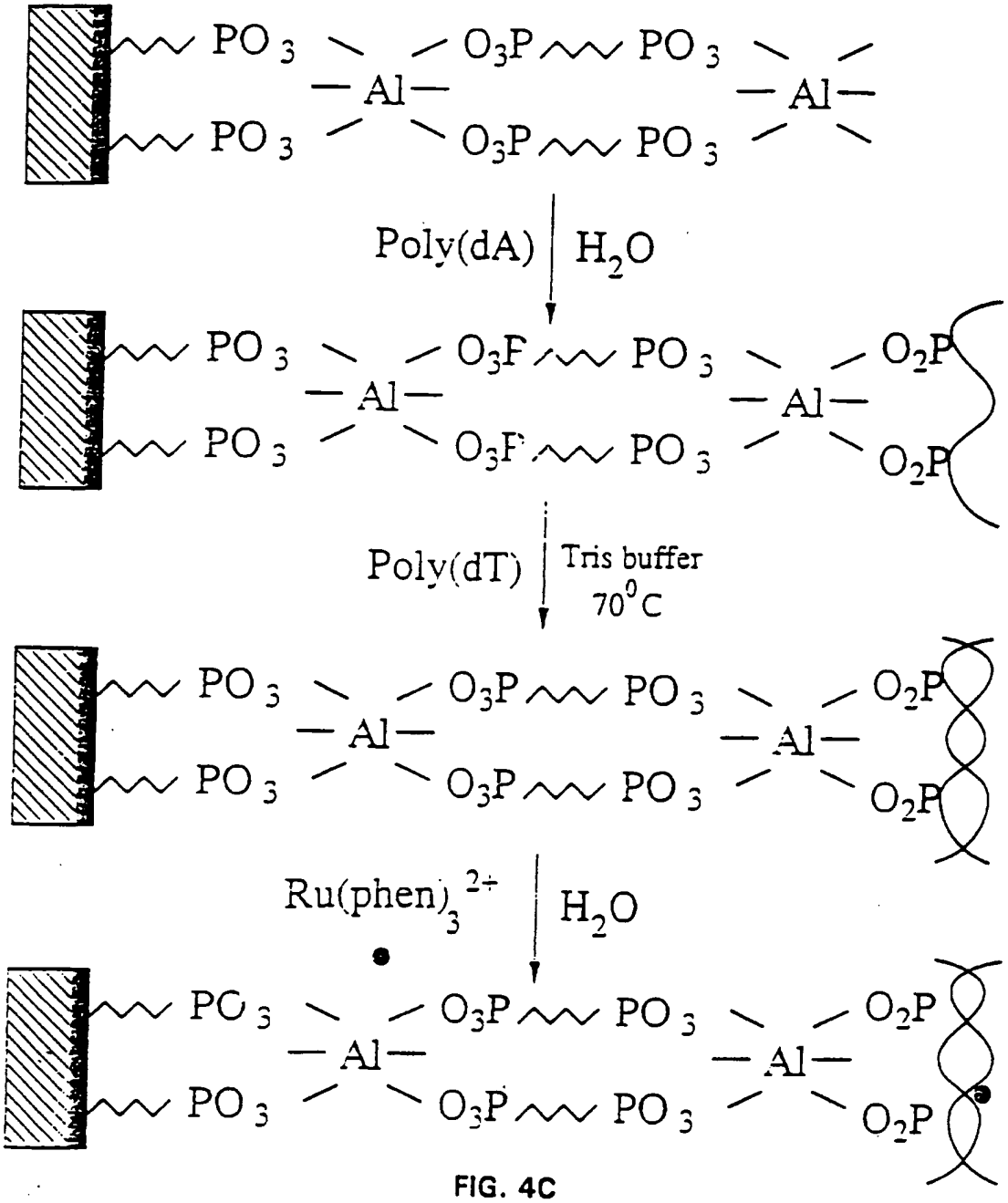

PO3
O3P
PO3
Al
Al
PO3
O3P
PO3
Poly(dA)
H2O
PO3
O3F
PO3
Al
Al
O2P
PO3
O3F
PO3
O2P
Poly(dT)
Tris buffer
70°C
PO3
O3P
PO3
Al
Al
O2P
PO3
O3P
PO3
O2P
Ru(phen)3 2+
H2O
PO3
O3P
PO3
Al
Al
O2P
PO3
O3P
PO3
O2P

FIG. 4C

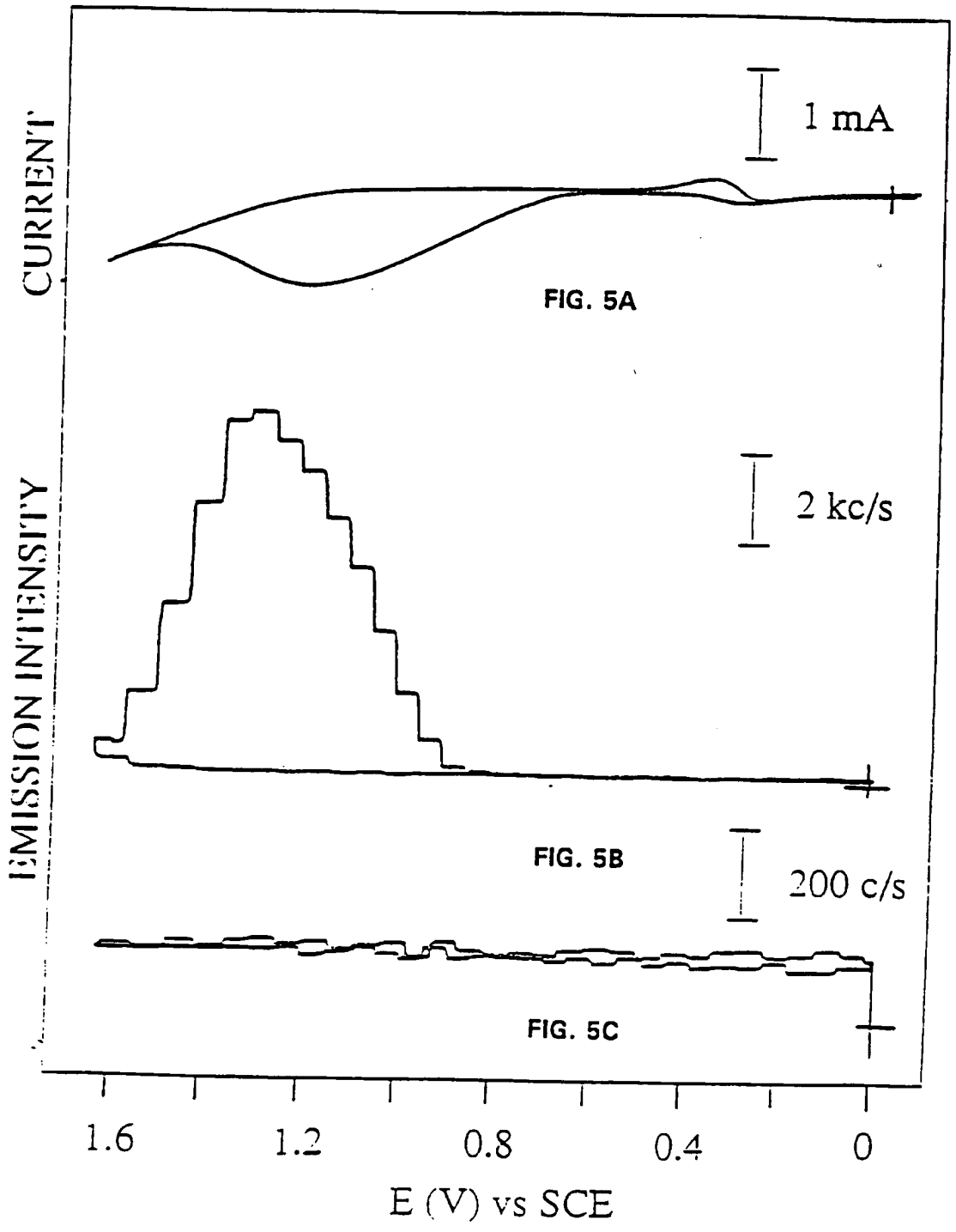


FIG. 5A

FIG. 5B

FIG. 5C

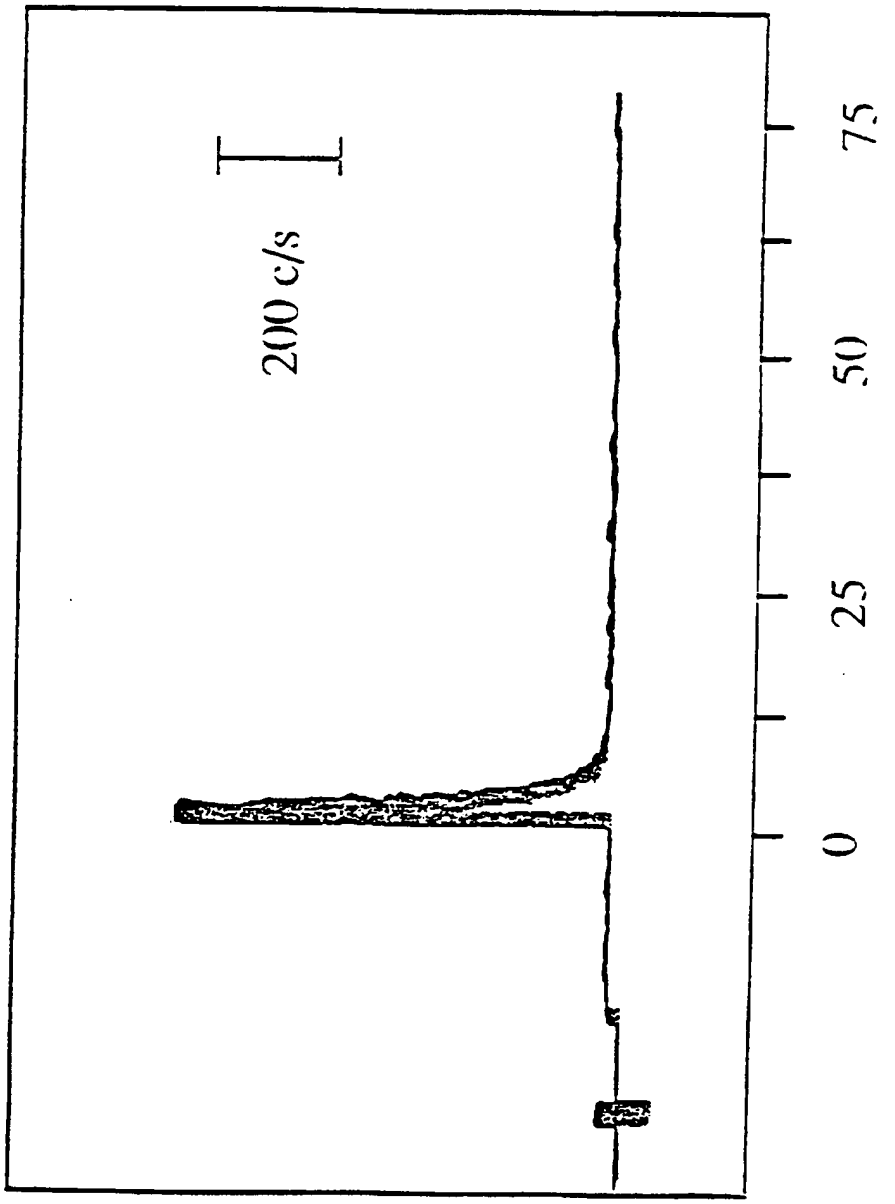


FIG. 6

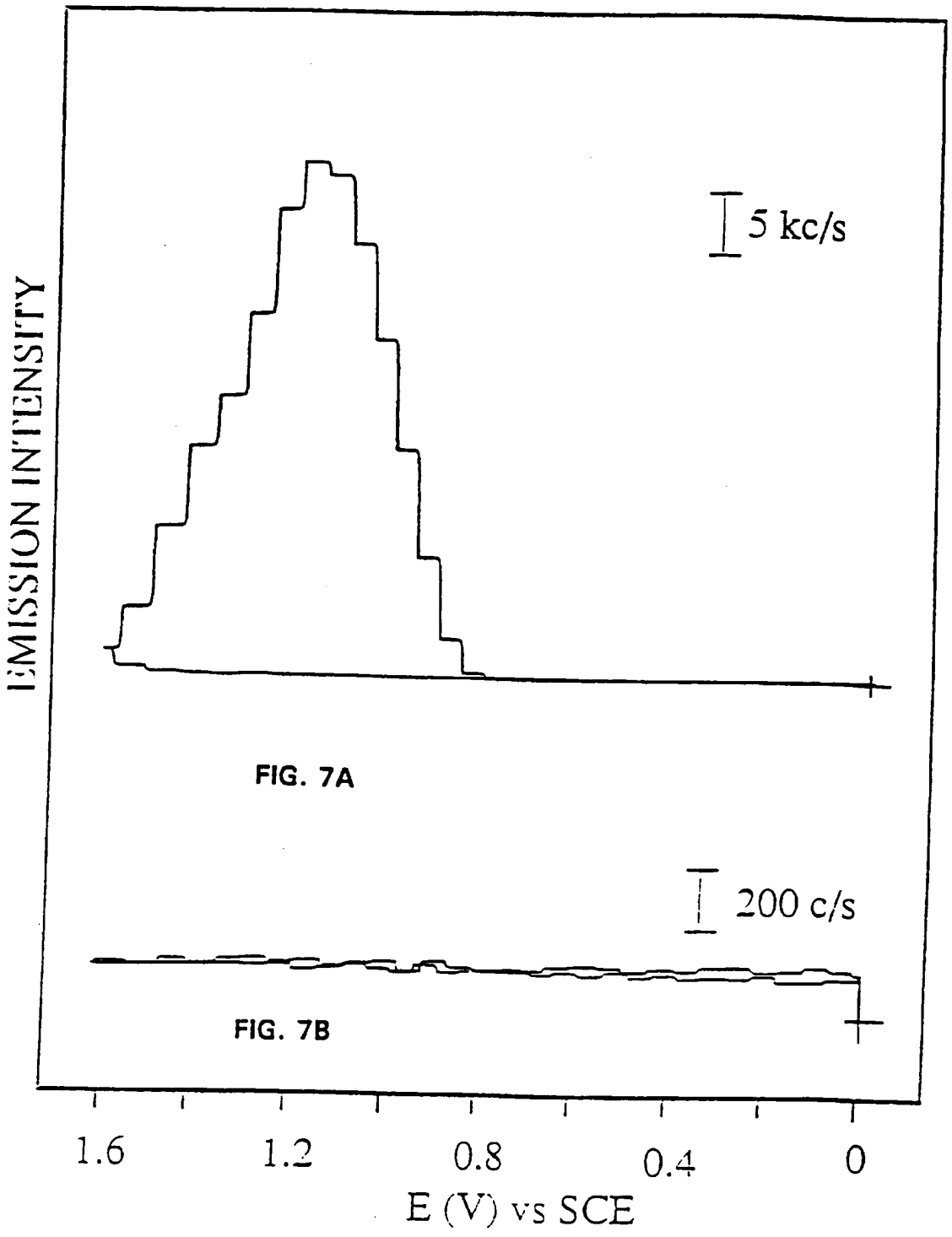


FIG. 7A

FIG. 7B

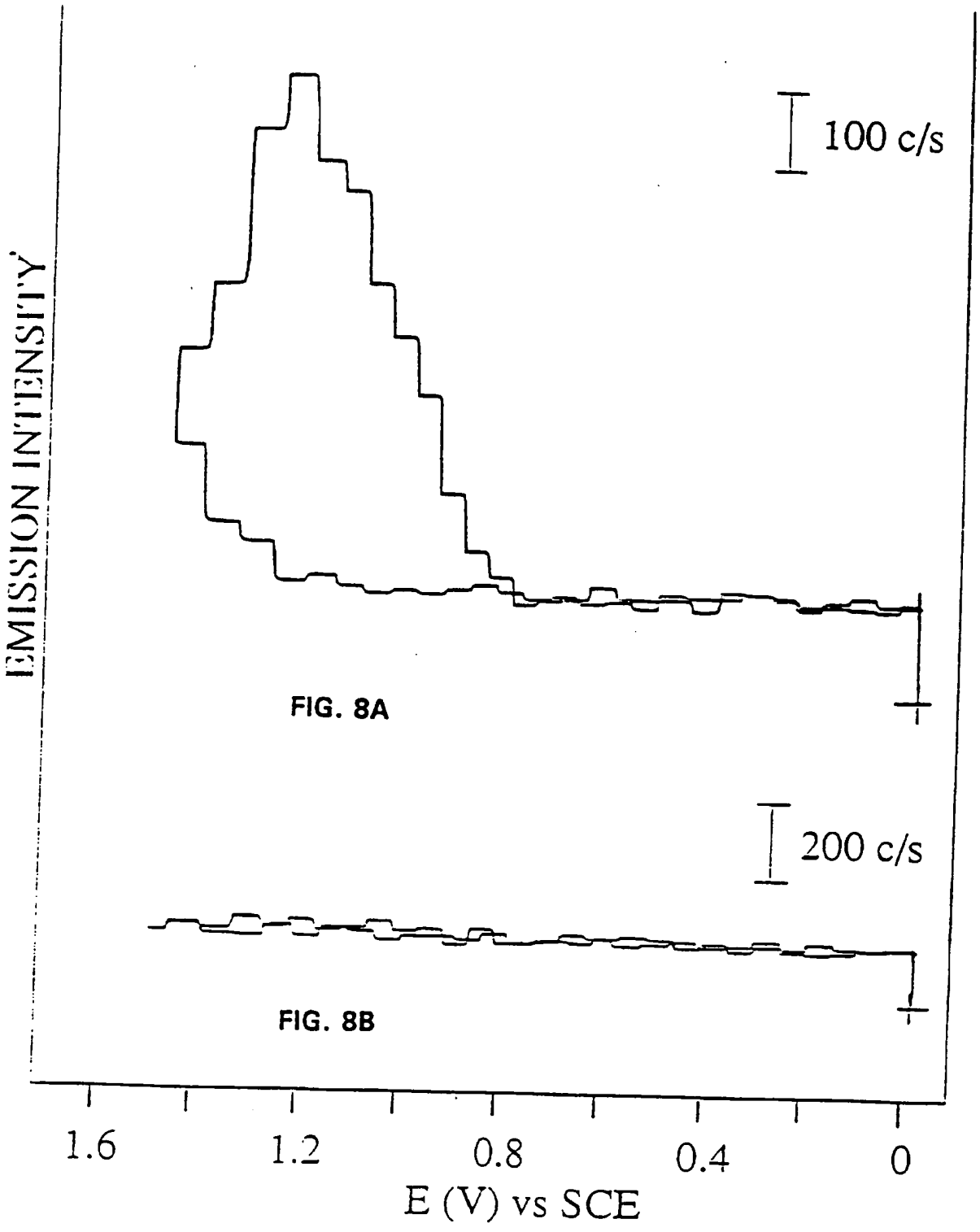


FIG. 8A

FIG. 8B

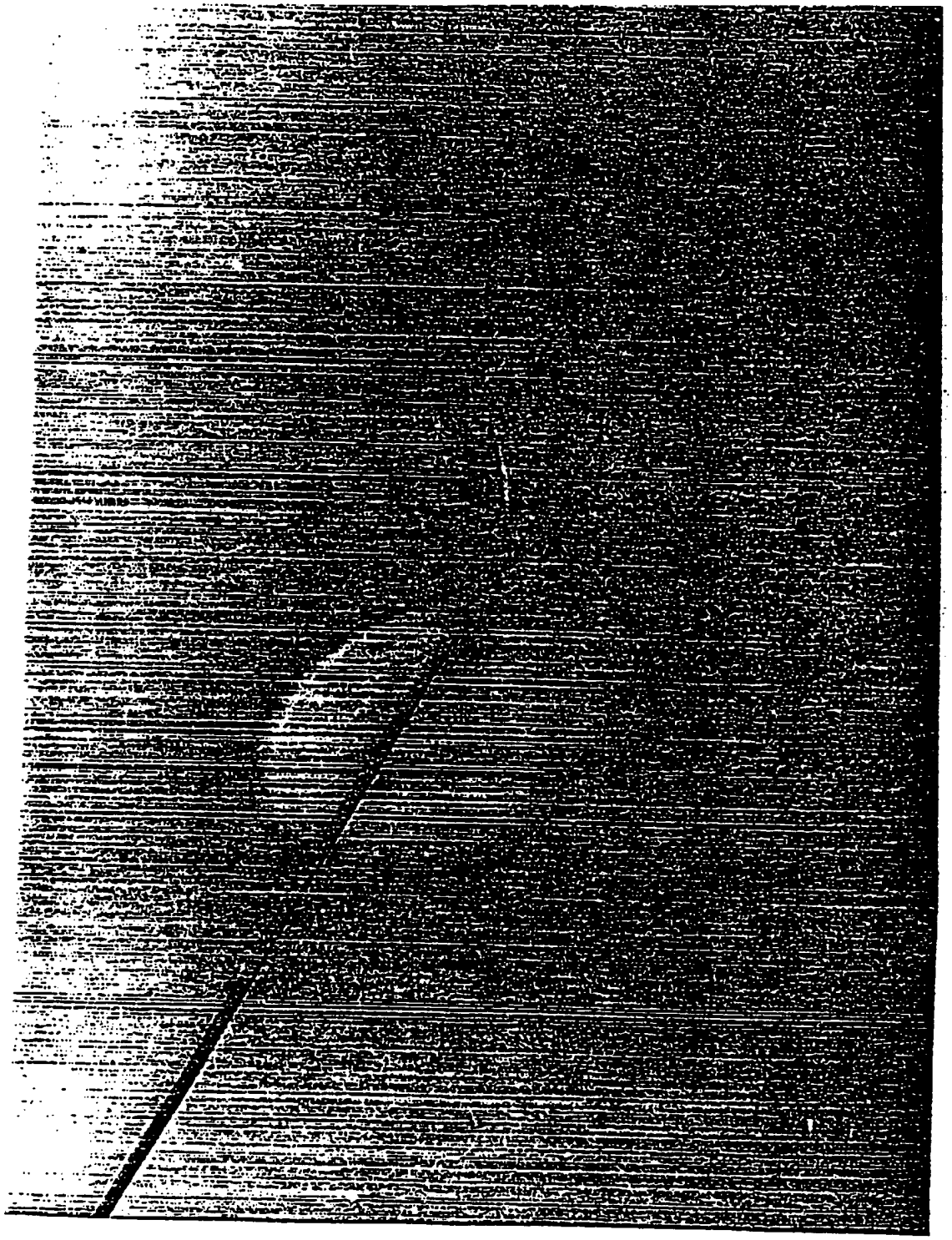


FIG. 9A

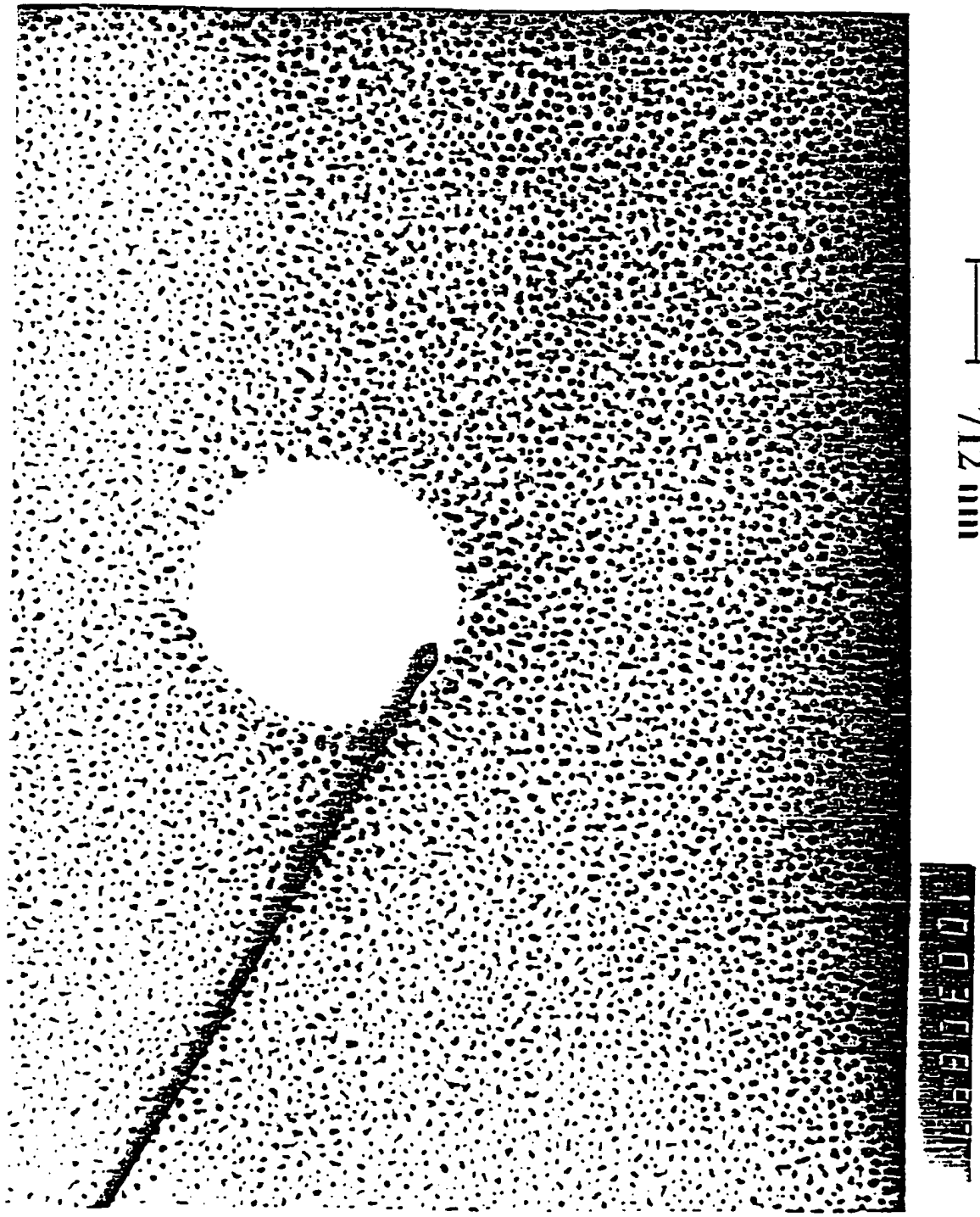
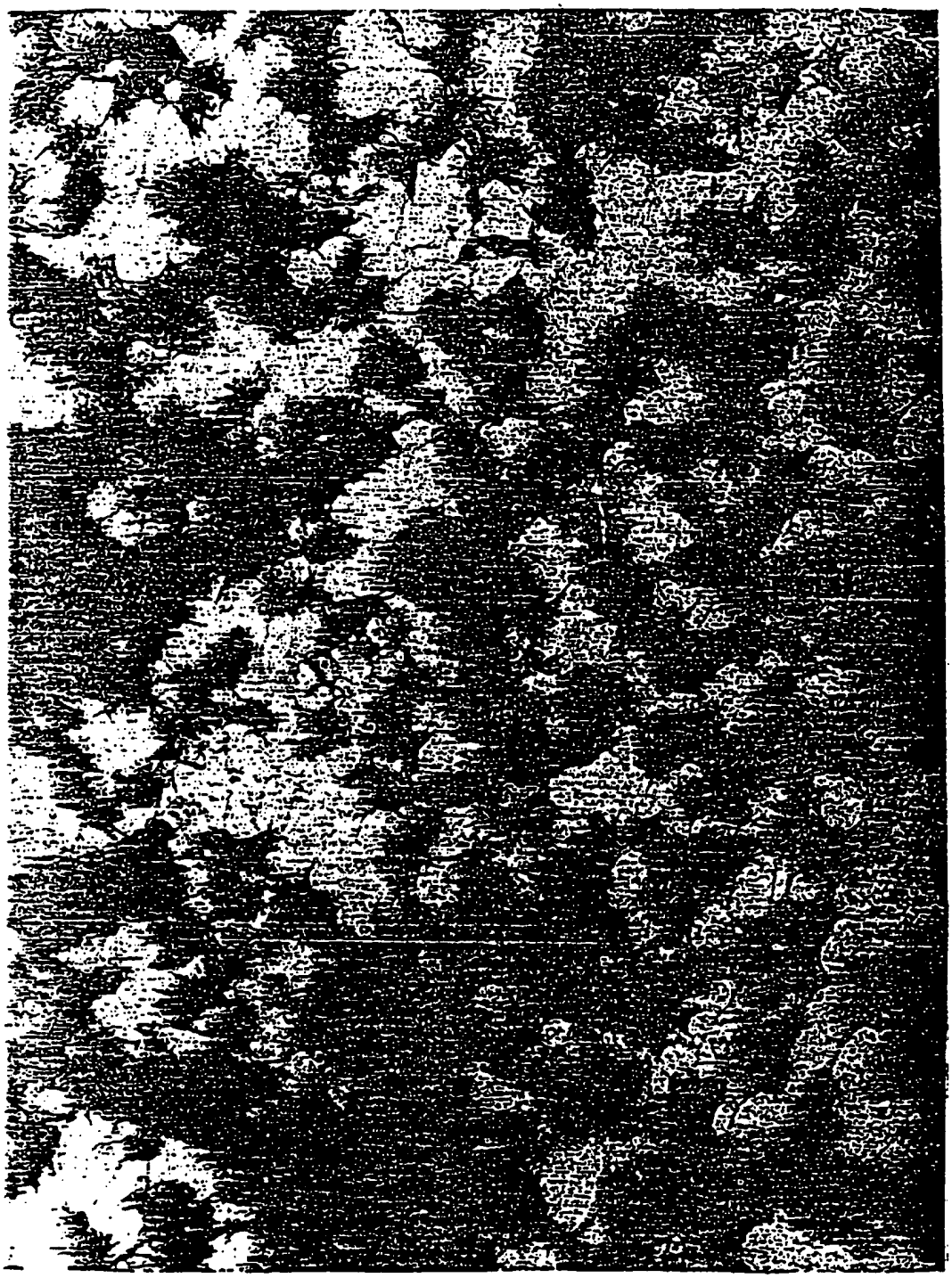


FIG. 9B



712 mm

FIG. 9C

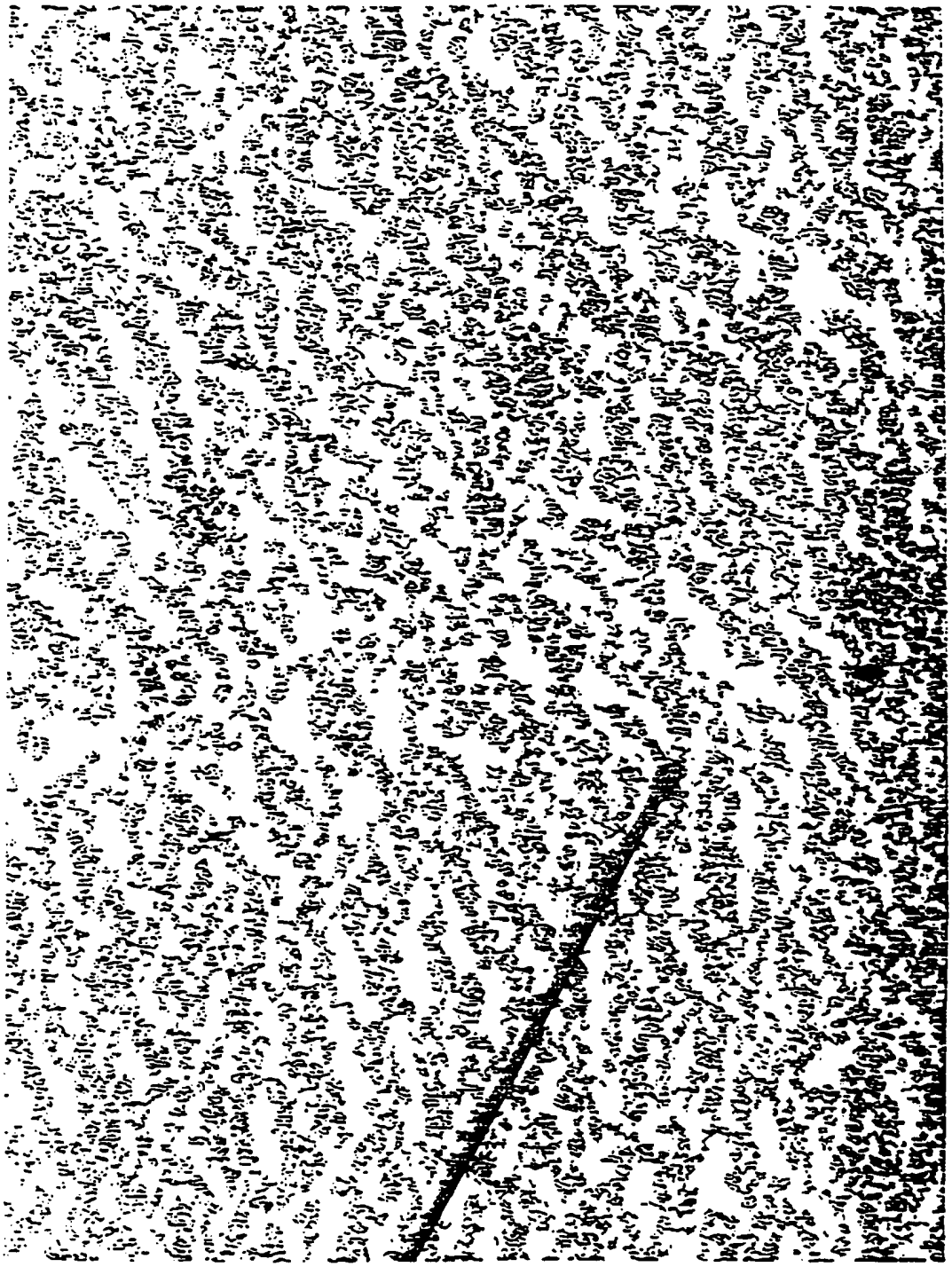


FIG. 10A

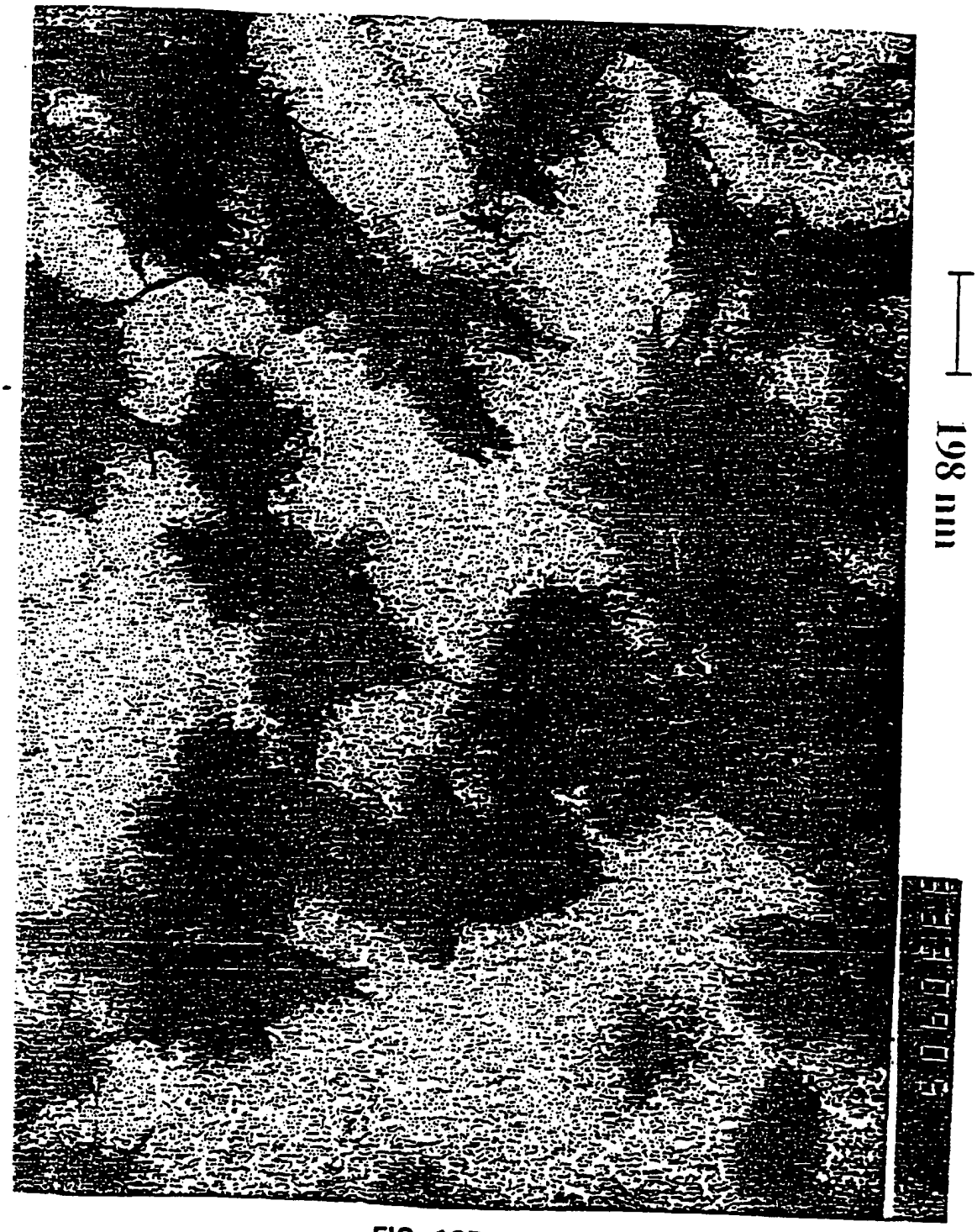


FIG. 10B

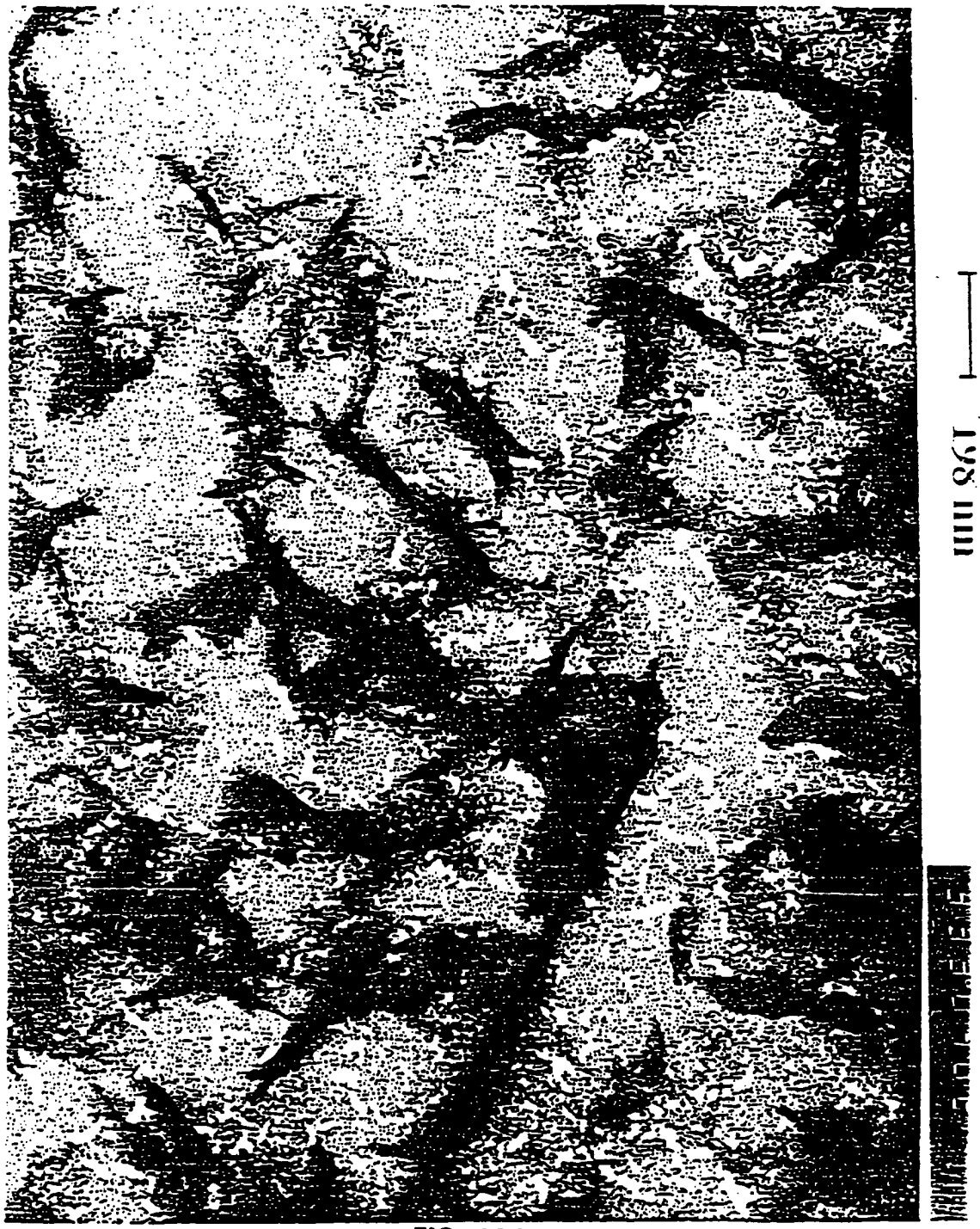


FIG. 10C

SEQUENCING VIA ARRAY HYBRIDIZATION

1. Form array by surface attachment of complete set of oligonucleotide probes (as above)

FIG 11A

2. Expose to DNA to be sequenced. Detect via ECL as above to recognize zones with complementary sequence.

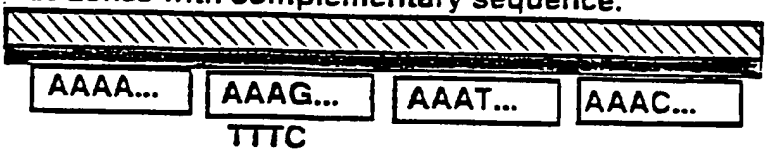


FIG. 11B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/10630

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :435/ 4, 5, 6, 7.1, 91.2; 536/24.3; 205/175;
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/ 4, 5, 6, 7.1, 91.2; 536/24.3; 205/175;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Analytical Chemistry, Volume 66, Number 6, issued 15 March 1994 , H. Su et al, "Interfacial Nucleic Acid Hybridization Studied by Random Primer 32P Labeling and Liquid-Phase Acoustic Network Analysis", pages 769-77, see Figure 2 and page 771, column 2, paragraph 3 and 4.	19
Y	WO 93/22678 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 11 November 1993, pages 3-5.	1-20
Y	US, A, 5,238,808 (BARD ET AL) 24 August 1993, column 5, lines 59-70, column 6.	1-20
Y	US, A, 3,591,838 (FUJIWARA ET AL) 06 July 1971, column 1, lines 48-58 .	13-18

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 OCTOBER 1995	Date of mailing of the international search report 26OCT1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DIANNE REES Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/10630

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,124,022 (EVANS, II ET AL) 23 June 1992, column 5, lines 48-59.	1-20
A	US, A, 5019,343 (HWA ET AL) 28 May 1991, column 3, lines 1-32.	1-20

INTERNATIONAL SEARCH REPORT

Int...ational application No.
PCT/US95/10630

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12Q 1/00, 1/70, 1/68; C 12 P 19/34; C07H 21/04; C25D 11/12; G01N 33/53

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, AIDSLINE, ANABSTR, AQUASCI, BIOBUSINESS, BIOSIS, BIOTECHABS, BIOTECHDS, CAPLUS, CEABA, EMBASE, JICST-EPLUS, LIFESCI, MEDLINE, PHIN, PROMT, SCISEARCH, TOXLINE,TOXLIT,USPATFULL,WPIDS
Search terms: biosensor, electrogenerated, ecl, electrochemiluminescence, alkanebisphosphonate, electrodes, chelators, polydentate, osmium or ruthenium labels,nucleic acid hybridization, immunoassays.